"Study on drug standardization of *Shallki Niryasa* [Oleo Gum resin] and *Twak Churna* [Bark powder]"

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AYURVEDA VACHASPATI - M.D (AYU)
IN
DRAVYAGUNA VIGYAN

BY

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CERTIFICATE

This is to certify, according to the ordinance of regulations to the degree of Doctor of Medicine M.D (Dravyaguna Vigyan), this thesis titled **"Study on drug standardization of *Shallki Niryasa* [Gum resin] *and Twak Churna* [Bark powder]"** is submitted to Swami Ramanand Teerth Marathwada University as a partial fulfillment of the requirements for the final examination of Ayurved Vachaspati [Doctor of Medicine] M.D (Ayu) in Dravyaguna vigyan.

I certify that **Dr. Thrigulla Saketh Ram** has done drug standardization study under my guidance during the academic period 2000-2002. This thesis was written after a series of scientific discussions on the subject, the drug study, observations and results are genuine.

I therefore recommend this thesis to be submitted to the adjudicators

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❋ नमामि धन्वंतरिमादिदैवं सुरासुरैर्वन्दितपादपद्मम् ।
लोकेजरारुग्भयरोगनाशं दातारमीशं विविधौषदीनां ।।

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15.	Graphical representation of Quantitative values Sheet –3.	Do

Abbreviations mentioned for the Texts Referred.

Ch.Su	Charaka Sutrasthana
Ch.Vi	Charaka Vimana sthana
Su.Su.	Susruta Sutra Sthana
AS. Su	Astanga Sangraha Sutra Sthana
R.N	Raj Nighantu
K.N	Kayyadeva Nighantu
S.N	Shadrasa Nighantu [Abhidhana ratnamala]
M.N	Madanapala Nighantu
C.S	Charaka Samhita
S.S	Susrutha Samhita
A.S	Astanga samgraha
D.N	Dhanvantari Nighantu
B.N	Bhavaprakash Nighantu
N.R	Nighantu Ratnakar
G.N	Gada Nigraha
KO	Aiyer Nambodiri, A.N., Kolammal. M, Pharmacognosy of Ayurvedic Drugs .
AR	Alix Taisom.,- Harita Samhia, French Institute, Pondicherry.
AH	Astanga Hridaya Kosha.
AP	The Ayurvedic Pharmacopoeia of India.
AF	Ayurvedic formulary of India .
GW	George Watt. A Dictonary of the Economic Products of India, Vol I- VA.
KB	Kirtikar , Basu, Indian Medicinal Plants (Revised By Blatter, Acius & Mhaskar)
KR	Kurup, P.N.V et al , Handbook of medicinal plants
MS	Modern Sheriff. . A Catalouge of Indian Synonyms.
NS	Mooss, N.S., Single Drug Remedies, Kottayam.
NM	Mooss, N.S Ganatas of Vahata.
IP	Indian Pharmaceutical Codex.
KM	Nadkarni ,Indian Materia Medica
VA	Raghnath Iyer, Inidan Medicinal plants .
PV	Sharma PV , Dravyaguna Vignana,
SI	Sivarajan, Indira Balachandran, Ayurvedic Drugs and Their plant sources.
BC	Thakur Balwant Singh & Chunekar, K.C,Glossary of Vegetable Drugs in Brihatrayi.
VB	Vaidya Bapalal,Nighantu Adarsh.
BL	Vaidya Bapalal,Some Controversial Drugs of India.
DN	Vaidya Kamat, Studies on Meidicinal Plants in Dhanvantariya Nighantu.
WH	Whitelaw Ainslie, Materia Indica.

Introduction

"यो देहमर्पयति चान्यसुखस्य हेतोः
तस्मै वदान्यगुरवे तरवे नमोस्तुते"

भवभूति

Present era is the **"era of globalization "**for *Ayurveda*. Ever increasing popularity amongst all sections of people around the globe has brought in some essential changes in Ayurvedic practice. The multifold increase in demand for Ayurvedic preparations has made it obligatory for mass production. Indian Ayurvedic pharmaceutical industry is undergoing serious retrospection and change today, after GMP[Good manufacturing practices] and drug standardization of medicines has been made as mandatory requirements for licensing by Govt. of India[1] .

With the increase in commercial manufacturing there arose a need for increased for material inputs and mechanization. This led to more and more dependence on Business houses and others involved in the manufacturing of Ayurvedic drugs, on the suppliers of raw materials, who in turn took the help of local laborers to get the raw material collected from different parts of the country irrespective of the concentration of active principles and appropriate season of collection of a particular herb.

Sometimes because of consideration of commercial gains, adulteration, and substitution also become the name of the game. This phenomenon has alerted the industry, the academic and research institutions and the Government agencies leading to frantic efforts for standardization of raw materials and finished products.

The eagerness of providing quality product gave rise to establishment of R&D sections in various pharmaceutical companies. With growing interest in herbal drugs and Ayurveda all over the world in the present decade new channels, to export the drugs have opened up and with them have also risen the awareness towards standardization of Ayurvedic drugs[2].

Drug Standardization –Indian Scenario-legal status:

The Govt. of India, Ministry of health and family welfare appreciated the need to prescribe quality standards for Ayurveda drugs and Drugs and cosmetics act-1940 was amended in 1964 to bring with in its purview the drugs of Ayurveda, Siddha, and Unani systems of medicine.

The implementation of the Drugs Act and the rules there under, it was considered necessary to work out standards for establishing the quality of these drugs.

The Pharmacopoeia Laboratory of Indian Medicine (PLIM),Ghaziabad has been constantly engaged since 1970 in developing standards of quality, purity and strength of Ayurvedic drugs. This Laboratory is guided by the Ayurveda Pharmacopoeia Committee (APC) having experts of Ayurveda, Pharmacognosy, chemistry and pharmacy. On the advise of APC the Pharmacopoeia Laboratory of Indian Medicine (PLIM), has been carrying scientific work to generate the data on various Monographs of drugs[3].

Government of India has issued guidelines to the pharmaceutical companies to generate and Standards for their products and adopt GMP good manufacturing practices to ensure quality of medicines they prepare.

Rationale of drug Standardization:

Selecting genuine raw material, good manufacturing methods, abiding by the spirit of traditional practices are essential components of drug standardization. The aspects of drug standardization becomes very important for the traditional systems such as Ayurveda where herbal drugs are extensively used.

The multiplicity and the wide geographical variations which are characteristic features of India and its medical system "Ayurveda" make it a vulnerable system which can be misused if not properly documented. Standardization of the medicinal plants mentioned in the texts will provide the essential credibility for their use in the native land, and will help in increasing the global acceptance also.

Definitions of Standardization[4]:

Encyclopedia Britannica defines Standardization as:

1. 'The process of developing and bringing about the utilization of Standards'
2. A standard is defined as "a **numerical value** which quantify the parameter and thus denotes quality and purity of a material".
3. 'A **standard** is that which has been selected as a model to which objects or actions may be compared'

4. Product standard: 'intended to describe attributes and ingredients of manufactured item and embodied in drawings, formulas, material lists, descriptions or models.'
5. Product standard for processes, methods, operation and equipment included in technical specifications, inspection and test methods, machine set up, drawing and operation sheets. '

Aim of Standardization[5]:

Standardization has to be aimed at –

a. Raw material

- Identification of the proper drug
- Identification of adulterants and substitutes
- Quality and purity of the herb Efficacy of the drug
- chemical composition
- Determination of active principle

b. Process standardization

- Cleaning procedure Grinding procedure
- Fermentation procedure Extraction procedure
- Storing (chopping etc.,) procedure Handling procedure.

c. Finished product

- Physical characteristic Chemical characteristics
- Micro-biological characteristics Stability
- Appropriate packing

Approach For Drug Standardization:

Standardization of raw material should consist of :

Pharmacognostic identification.

Identification of suitable marker compounds which may or may not be active principles

Ecological mapping of medicinal plants, Pharmacological efficacy, toxicity

Agronomic aspects of cultivation etc.

Selection of the Drug:

Guggulu forms the bulk of Ayurvedic formulations which are meant for treating various Vata and Kapha Vikaras. Interestingly in India we come across and use different types of Oleo-gum-resins such as Kan guggul, Salai guggul, Hira bol, Sarjars etc.

Amongst these two most important products come from the Burseraseae family i.e. Commiphora mukul and Boswellia serrata which yield Kan Guggulu and Salai guggul respectively. Amongst these two the former is used widely and also studied thoroughly . It finds its uses as an effective anti-inflammatory agent, as well as an anti-cholesterolaemic drug. Unfortunately the Commiphora species is under threat due to excessive tapping and its use as fuel by people especially in Rajasthan region. This situation has warranted to search for a viable alternative for Guggulu and Salai guggul finds a quick place with similar effectiveness as an anti inflammatory agent and equally good lipid lowering drug.

Shallaki Guggulu –Most happening Ayurvedic Drug:

The unique quality of Leucotriene [inflammatory mediators of lipoxyginase cycle] inhibiting activity[6] of Shallaki [Boswellic acids] has made it a special drug. The clinical studies also have shown considerable anti arthritic activity which has increased its demand tremendously. Many Pharmaceutical companies are marketing it either as a single herb or as a compound preparation.

Importance of *Twak* and *Twak churna.*

References from *Susrutha Samhita* and *Charaka Samhita* imply that apart from the use of *niryasa* [gum resin] , use of *twak* and *twak churna* [bark &bark powder] is also recommended for treating conditions like- upadamsha vrana[7], swasa vyadhi[8] which speak about the efficacy and utility of twak churna. In spite of the availability of this drug usage of twak churna is seldom put to use by any vaidya. With due consideration for usage of *Shallaki twak churna* and the possible scope of its utility as substitute for *niryasa*, it has been strongly felt that developing standards for *twak* and *twak churna* are essential, which will benefit the future clinical work .

Considering these key issues it has been felt and a humble effort has been made to develop standard parameters for the plant *Shallaki niryas*,and *twak churna* . This will try to remove ambiguity and ensure the purity and strength of the material for collection of genuine samples of *Shallaki Niryas*, *twak churna* and their utilization.

References:

1. Dr. M.D. Nair, Standardization imbroligo effects traditional medicinal products, Heritage Healing, Sep 2000, healthcare communications(p) ltd. #8, 5th cross Extn. Bhaktavathsalam Nagar, Adayar, Chenni-20 (India) p.20
   [Notification: Ministry of health and family welfare, dept. of ISM, Jan 23, 2000-amendment to Drugs & Cosmetics rules, 1945 (rules 155B-157B-schedule-T).

2. C.K.Katiyar,A.Vittal babu,D.B.Narayana-Standardization of ayurvedic drugs-Problems,Realities and rationale; dabur Inida Ltd., Dabur Research foundation.,2,site-iv,Sahibabad,Ghaziabad-201 010 (U.P).- Traditional medicine.,Oxford &IBH co.Pvt.Ltd. 1993.,p.328

3. Mrs.Shailaja Chandra.,Forward.,The Ayurvedic Pharacopoeia of India- III,NISCOM,New Delhi,1999.

4. C.K.Katiyar,A.Vittal babu,D.B.Narayana-Standardization of ayurvedic drugs-Problems,Realities and rationale; dabur Inida Ltd., Dabur Research foundation.,2,site-iv,Sahibabad,Ghaziabad-201 010 (U.P).- Traditional medicine.,Oxford &IBH co.Pvt.Ltd. 1993.,p.328
5. ibid.,p.329

6. G.B. Singh et.al., Anti-inflammatory action of boswellic acids, Phytomedicine, vol-3, Gustav Fisher Verlag, Stuttgart, Jena, Newyork, 1996, p. 81-85.

7. Susruta Samhita with Nibandha Sangraha commentary, chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint, 1994,p.368

8. Charaka Samhita with Ayurveda Deepika commentary, Chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint, 1992, p.436 -438.

2. Review of Literature

There has not much clinical and other types of research is recorded from the ayurvedic faculty regarding this drug. There are numerous works published in various reputed journals regarding various aspects of research based on the plant *Shallaki* Boswellia serrata Roxb. and allied species. Here an attempt has been made to categorize and present the various works to this date.

A. Clinical/medical research

1. Antibacterial activity of some essential oils [Cinnamomum tamala, Bossella serrata Roxb. Nordostachys jatamansi]. , Girgune-JB, Jain-NK; Garg-BD. , Holkar Science College, Indore, India. Current Science . 1978, 47:13, 454-455;
2. Rao-DR; Kaur-A; Effect of the essential oil from the gum oleoresin of Boswella serrata Roxb. On the gonads of male Dysdercus similis F. Dept. of Zoology , Osmania University, Hyderabad –7, India, Current Science . 1989, 58; 14, 822-824;
3. Kulkarni-RR; Parki-PS; Jog-VP, Grandage-SG; Bhushan-Patwardhan-P; Treatment of Osteoarthritis with a herbomineral formulation ; a double-blind, placebo-controlled, cross- over study [Stem of B. serrata is used]., Univrsity of Poona, Ganeshkhind, Pune-7, India ; Journal of Ethnopharmacology, 199133; 1-2, 91-95;
4. Mack-T; Ammon-HPT; Safayhi-H; Inhibition of leukotriene generation by a gum resin esudate of Boswella serrata in rat peritoneal leukicytes; Pharmazeutisches Institut, Lehrstuhl Pharmakolotie, Universtat Tubingen, 7400, tubingen, Germany. Planta-Medica. 1990. 56; 6, 662; paper presented at the Symposium on biology and Chemistry of active natural substances, Bonn, Germany, 17-22 Nuly 1990;
5. Ammon-HPT; Safahai-H; Mack-T; Sabieraj-j; samulsson-G; Department of pharmacoloty, Instute of Pharmaceutical Sciences, Eberhard-Karls University, D-7400, Tubingen, Germany; Mechanixm of anti inflammatory actions of curcumine and boswellic acids. , Second international congress on ethnopharmacology, Uppasala, Sweden, 2-4 July 1992. Journal of Ethnopharmacology. 1993, 38; 2-3, 113-119;

6. Duwiejua-M; Zeitlin-IJ; Waterman-PG; Chapman-J; Mhango-GJ; Provan-GJ; dept. of Physiologyand Pharmacology, royal college, University of Strathclyde, Glasgow GI 18 W, Scotland; Anti flammatory activity of resins from some species of the plant family Burseraceae. ; Planta-Medica. 1993, 59:1, 12-16;
7. Singh-GB; Surjeet-singh; Sarang-Bni; Singh-S; Bani-S; Regional Research Laboratory, Canal Road, Jammu-Tawi –1, India; Alcoholic estract of Salai-guggul ex-Boswellia serrata, a new natural source NSAID. – Drugs of today 1996, 32:2, 109-112;
8. Safayhi-H; Sailer-ER; Ammon-HPT; Department of Pharmacology, Institute of Pharmaceutical sciences, University of Tueningen, Auf der Morgenstelle 8, D-72076 tuebingen, Germany. Symposium on Salai guggual –Boswellia serrata, Halle (Saale), Germany 3-7 September, 1995. Phytomedicine. 1996, 3:1, 71-72;
9. Rall-B; Ammon-HPT; Sarayhi-H; Dept. of Pharmacology, Institute for Pharmaceutical Sciences, University of Tuebingen, Auf der Morgenstelle 8, D-72076 tuebingen, Germany; Symposium on Salai guggal –Boswellia serrata, Halle (Saale); Germany 3-7 Sep. 1995. Phytomedicine. 1996, 3: 1, 75-76;
10. Knau-U; Wagner-H; Dept. of Immunology, the Scripps Research Institute, 10666, North Torrey Pines Road, La Jolla, CA 92037, USA. -Effects of boswellic acids of Boswellia serata and other triterpenic acids on the complement system ; Symposium on Salai guggual –Boswellia serrata, Halle (Saale), Germany 3-7 September, 1995. Phytomedicine. 1996, 3:1, 77-81;
11. Singh-GB; Surjeet-singh; Sarang-Bni; Singh-S; Bani-S; Regional Research Laboratory, Canal Road, Jammu-Tawi –1, India- Anti inflammatory actions of boswellic acids. Symposium on Salai guggual –Boswellia serrata, Halle (Saale), Germany 3-7 September, 1995. Phytomedicine. 1996, 3:1, 81-85;
12. Singh-GB; Surjeet-singh; Sarang-Bni; Singh-S; Bani-S; Regional Research Laboratory, Canal Road, Jammu-Tawi –1, Indi-Toxicity and sagety evaluation of boswellic acids - Symposium on Salai guggual –Boswellia serrata, Halle (Saale), Germany 3-7 September, 1995. Phytomedicine. 1996, 3:1, 91-94.
13. Etzel-r; Karl-Theodor-Str. 14, D-82343, poecking, Germany. Special extract of Boswellia serrata (H15) in the treatment of rheumatoid arthritis. Symposium on Salai guggual –Boswellia serrata, Halle (Saale), Germany 3-7 September, 1995. Phytomedicine. 1996, 3:1, 91-94

14. Ammon-HPT- Dept. of Pharmacology, Institute for Pharmaceutical Sciences, University of Tuebingen, Auf der Morgenstelle 8, D-72076 tuebingen, Germany - Salai guggal –Boswellia serrata ; from a herbal drug to a specific inhibitor of leukotriene biosynthesis. Symposium on Salai guggual –Boswellia serrata, Halle (Saale), Germany 3-7 September, 1995. Phytomedicine. 1996, 3:1, 67-70;
15. Savita-Chaurasia; Tripathi-P; Tripathi-YB; Chaurasia-S; dept. of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, India. Antioxidant and anti-inflammatory property of Sandhika: a compound herbal drug. Indian Journal of Experimental Biology. 1995, 33: 6, 428-432;
16. Safayhi-H; Boden-SE; Schweizer-S; Ammon-HPT; Dept. of Pharmacology, Institute for Pharmaceutical Sciences, University of Tuebingen, Auf der Morgenstelle 8, D-72076 Tuebingen, Germany. Planta-Medica. 2000, 66: 2, 110-113;
17. Shao-Yu; Ho-Chi Tang; Chin-Cheekok; Badmaev-V; Ma-Wei; Huang-Moutuan; Shao-Y; Ho-Cr; Chin-CK; Ma0W; Huang-MT; Dept of plant science, Cook College, Rutgers, State University of New Jersey, New Brunswick, NJ 08903, USA. - Inhibitory activity of boswellic acids from Boswellia –serrata against human leukemia HL-60 cells in culture. Planta medica. 1998, 64: 4, 328-331;
18. Trubestein-G; An der Heppenmauer 10, D-63619 Bad or b, Germany. 1999. Effects of Salai guggal on RA;
19. Wildfeer-A; Neu-IS; Safahay-H Metzger-G; Wehrmann-M; Vogel-U Ammon-HPT; Dept. of Pathology, Medical faculty, university of Ulm, Universityof Tuningen, Germany; Effects of boswellic acids extracted from a hernal medicine on the biosynthesis of leukotrienes and the course of experimental autoimmune encephalomyelitis. Arzneimittel-forschung. 1998., 48: 6, 668-674:

B. Forest research

1. Garg-RK, Vyas-LN, Litter production in deciduous forest near Udaipur (South Rajasthan), India;tropical –ecological-systems 1975,131-135
2. Garg-RK, Vyas-LN,vyas-NL ,Litter production and nutrient release in deciduous forest of Bansi, Udaipur, India.Flora-german-Democratic-Republic. 1976. 165:2

C. Cultivation and Harvesting:

1. Murthy-TK; Shiva-MP;Salaiguggul from Boswellia serrata Roxb. –Its exploitation and utilization forest Research Institute and Coll., Dehra Dun. , India U.P. , 1977103:7, 466-474;12,
2. Hofmann-TR,Growing and propagation of frankincense (Boswellia carteri); brooklyn Botanic Garden, NY 11225. ,USA. , Plan propagator. 1986, 32:2, 11-13;
3. Sarin-YK; Atal-CK., Indian Olibanum as perfumery raw material Regional research laboratory, Jammu-Tawi, India, Pafai-Journal. 1982, 4:2, 13-18;
4. Barber-C., The frankincense tree [Boswellia sacra and B.frreana]in somalia; propositions for propagatio and management, Laboratorie Botanique, Institut Botanique, 43000 Montpellier, France. 1984, I+28 pp.
5. Chazee-L , the collection of Frankincense. Part 4.1987, iv+ 42 pp.
6. Farah-AY,The milk of Boswella forests; frankincense production among the pastoral Somali, Somali academy of sciences, Mogadishu, Somalia, 1988, 382 pp.
7. Prakash-DVSSR; Suresh-Chand-S; Kishor-PBK, In vitro response from xultured anthers of Boswella serrata Roxb. (Burseraceae)] . , Pant Tissue culture and Genetics Research Group, School Life Sciences, Devi Ahilya University, Khandwa Road Campus, Indore-452001, India, Plant tissue culture and biotechnology: emerging trends; Proceedings of a symposium held at Hyderabad; India 29=31 Jan, 1997. 1999, 226,-231;
8. Harries-HC., Economic harvesting of tropical tree crops. International Coconut Cultivator registration Authority, 17 Alexander road, Lodmoor Hill, Weymount, Dorset DT4 7 QQ, UK. Planter. 1998, 74; 864, 157-16u, 163-165;

D. Pharmacognosy

1. Dennish-TJ; Billore-KV; Mishra-KP; Pharmacognostic study on gum oleo-resin of Boswella serrata Roxb. Dept. of Chemistry . , Osmania Univ . , Hyderabad, India; Bulletin-of Medicc-ethno-Botanical-Research; 1981, 1:3, 253-260.
2. Nair-MNB; Subrahmanyam-SV . , Ultrastructure of epothelial cells of oleo-gumresin secretion in Boswellia serrata (Burseraceae). , Faculty of forestry, University Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia; IAWA – Journal. 1998, 19:4, 415-427;

5. ARA-C.Jing-YK;han-R;Combination induction of cell differentiation of HL-60 cells by daidzein (s86019) and BC-4 or Institute of material medica, Chinese academy of medical sciences, Beijing 100050, china.Acta-pharmaceutica-sinica. 1993,28: 1, 11-16;

6. Babita –mahajan ;taneja-sc; sethi-vk; dhar-kl; mahajan-b; Two triperpenoids from boswellia serrata gum resin.Natural products chemistry division regional research laboratory,Canal road jammu-tawi 180001,India.Phtochemistry . 1995,39:z, 453-455;

7. Babita –mahajan ;taneja-sc; sethi-vk; dhar-kl; mahajan-b; Structure-activity relationships of the nonredox –type, non-competetive leukotriene biosynthesis inhibitor, acetyl-11-keto-beta-boswellic acid.xDepartmrnt of pharmacology, institute of pharmaceutical sciences ,University of Tuebingen, auf der morgenstelle 8, d-72076.Tuebingen Germany; Symposium on salai guggal-boswellia serrata, halle(saale);Germany 3-7 september,1995. Phytomedicine.1996, 3:1,73-74;5

The ultrastructure of epithelial cells of loeo-gumresin ducts in Boswellia serrata, the source of indian olibanum [IF] is described.

D. Phyto-chemistry

1. Fattorusso-E; santacroce-C; xaasan-CF;4(23)- dihydroroburic acid from the resin (incense) of Boswelliacarterii [b.sacra],National Somali university, p.o. box 1081, Mogadishu, Somalia.Phytochemistry. 1983,zz:12,z868-z869;

2. Sharma-ra; varma-kc; Studies on gum obtained from boswellia serrata roxb.Dep. Pharmaceutical sci., univ. saugar, sagar, India; Indian-drugs. 1980, 17:8,225-227; chemical properties are reported of salai gum. Compared with tragacanth and CMC the gum is not a good suspending agent but emulsions prepared with it were slightly better than those from acacia.

3. Wahab-sma ; aboutabl-EA; E1-zalabani-SM; fouad-HA; pooter-hl-de; E1-fallaha-B;the essential oil of olibanum.Faculty of pharmacy, cairo university , 11562 cairo, Egypt.Planta-medica. 1987, 4, 382-384;

the essential oil of the oleogum resin from the bark of boswellia carterii[b.sacra] was prepared by direct steam distillaton and also by steam distillation of an n-hexane extract. Thirty-three components were identified; 11 of these (all minor constituents)were not found in the chromatogram of the oil from the n_hexane extract.the oils contain 62.1% easters, 15.4%easters alcohols, 9.9% monoterpene hydrocarbons and 7.1% diterpenes. Both oil preparations showed antimicrobial activity in screening tests,(that prepared by direct distillation being the more effective.) These findings support the use of the resin in local medicine to treat coughs and asthma.

4. Retamar-JA.IPNAYS(CONICET-UNL), chemistry and technology of natural and synthetic aromas.Santigo del estero z8z9,3000 santa Fe,Argentina, Monographias-de-la- academia-national-de-ciencias-exactas,-fisicas-y-naturales,-buenos-aries.1988,no.3,55-69;

Conceptual study

Part I

3. Conceptual Study Part - I

Ayurvedic perspective of Drug Standardization

The aim of Standardization is to ensure the uniformity of thought and practice so that the characteristic features of an entity is understood in the same manner by each and every one. Ayurveda as a separate wing of oriental learning has acquired all the qualities of a separate branch of knowledge whose purpose is the understanding the life [ayu- life; veda- knowledge] and allied aspects related to health and disease with respect to Human beings [स्वस्थस्य स्वास्थ्य रक्षणं - आतुरस्य विकार प्रशमनंच].

Ayurveda is a darshana in its own respect. It has combined the best elements of different Darshanas like Sankhya, Vaisheshika, Nyaya and gave them a practicable shape in the form Ayuveda. All this effort by innumerable number of Rrishis and vaidyas from a very remote period of history [nearly 5000 years down the lane] have given a standard out look and clearly entiled what is recognized as Ayurveda.

In Ayurvedic texts we find effors of standardization by different authors from Charaka Samhita to the latest texts. The standardization efforts can be classifed in to three principle groups:

1. **Standardization of Principles: This aspect covers uniform acceptance and documentation of basic principles** like

a. Panchamahabhut Theory, Tridosha [dosha, dhatu, mala, srotas, kala, garbha sharir etc.] siddhanta,
b. Hetu, Linga Aushad vignan
c. Rasapancak [rasa, guna, virya, vipak, prabhav/ karma] siddhant, samanya vishes siddhant etc.,
d. Aceeptance of pramana like Pratyaksha, Anumana, Shabda, Yukti, Upama etc., as tools of acquiring knowledge.

2. **Standard guideline followed for Classification of drugs as we find in Charaka sutra sthana- Bheshaja Chatuska [Therapeutic classification]** [1]

a. Vamana virechan kalpa, Dashemani etc.

b. Guna swarup classification- based on rasa – madhura skanda, amla skanda etc., based on swarupa (morphology) – mulini, phalini, kshirini, etc.

c. Based on the source of collection- jangam, oudbhid etc.,

3. **Kalpa – Formulation standardization**

a. Mana paribhasha [2]

b. Anukta dravya paribhasha,

c. Dravyagrahana, samrakshana vidhi, kala etc.,

d. Pratinidhi dravya vivaran

e. Pancha kashaya kalpana , vati, guti, asav, arista, ghrita, taila, lepa etc.

Dravyaguna shastra is a part and parcel of all ayurveda literature. It has assumed great significance in the present context as it brings together different aspects Pharmacotherapeutics. As Dravya forms the basis of ahara and aushadha it is imperative to understand the characteristics features of this branch and its applications in drug standardization;

The Characteristics of Dravyaguna Shastra

Dravyaguna shastra is that branch of knowledge that deals with the dravyas (drugs as well as diet) that help the maintenance of health and alleviation of disease in the purush (Human body) which is a concomitance of panchamahabhuta and atma (The soul or the consciousness). It also deals with the properitis, actions, dose, time of administration and various preparations of these dravyas.

The word dravyaguna includes the dravya as well as its qualities which imply the nature of the drug and provide a glimpse of its therapeutic and other actions.

Man has tried to make his life happier by using his intellect. For this, he made a big attempts to gain knowledge about the universe. He tried to derive logical conclusions about the genesis of the

universe. Through this exercise he realized that he himself is part of this universe which is made up of many visible and invisible components. There fore all the major and minor events of the universe have an influence on the human being. Once this was realized, man first started studying the basis of common properties. All existential phenomena were classified and a detailed and elaborate study of these was termed " Padarth vigyan" .Nothing was left out from this study. All phenomena of nature were included in it. Padarthvigyan means a study of all visible and invisible creations.

Dravyaguna shastra is developed as a part of padartha vigyan or can be called the therapeutic dimension of the same. The areas of Dravyaguna include the selection of the drug, studying its features for identification, nomenclature, physical properties , cultivation, collection, processing, methods of storage and the therapeutic uses. In the modern parlance the same is practiced in pharmacognosy and clinical pharmacology .

The concept of drug standardization and Ayurveda

Standards are meant for quality control which also includes the clinical efficacy and safety if it. Knowing a drug by its name, thorough understanding of its qualities and the effects on the body are a must for safe prescription of a drug. Following are the descriptions where we find a glimpse of development and utilization of standards from Ayurvedic Texts

A. Authority of Agamas (*Shastras*) as standard reference Texts.

अमीमांस्यान्यचिन्त्यानि प्रसिद्धानि स्वभावतः ।
आगमेनोपयोज्यानि विचक्षणैः ।। सु सू- 40.19
प्रत्यक्ष लक्षण फलाः प्रसिद्धाश्च स्वभावतः ।
नौषधीर्हेतुभिर्विद्वान परीक्षेत कथंचन ।। सु सू- 40.20
सहस्रेणापिहेतूनां नाम्बष्ठार्दिविरिचयेत् ।
तस्मात्तिष्ठेत् तु मतिमानागमे न तु हेतुषु ।। सु सू- 40.21

Acharyas of Ayurveda always give importance to the former Texts available to them which are nothing but the records of the previous scientific work and recommend the followers not to

indulge in any type of unnecessary discussion just for the sake of proving and for fun. It is always good for a physician to faith fully follow the authorized texts (*agamas*) to understand the therapeutic effects rather than just to indulge in discussion[6].

On a superficial level this discourse may seem very unscientific and discoraging for the question abilities of a scientific worker . but a close look at the statement clearly reveals that the ***Agamas*** are the documented facts of years of meticulous observation by **Nirajastama** individuals hence what ever they have recorded can be taken as standard and can be comfortably followed which definitely saves the valuable time and energy of the physician.

Even the modern trend follows the same principles of documenting and preparing national pharmacopoeias and national formularies which serve the purpose of *Agama* for the workers of the field.

B. Importance of dravya with respect to other pharmacological consideration like rasa etc.,

केचिदाचार्या बृवते द्रव्यं प्रधानं कस्मात ? व्यवस्थितत्वात्, इह खलु द्रव्यं व्यवस्थितं; न रसादयः यथा आमे फले ये रसादयस्ते पक्वे न सन्ति नित्यं हि द्रव्यमनित्या गुणाः , यथा कल्कादिप्रविभागः स एव संपन्नरसगन्धो व्यापन्नरसगन्धो वा भवतिः , स्वजात्यवस्थानाच्च, यथा हि पार्थिवं द्रव्यमन्यभावं न गच्छत्येवं शेषाणि, पंचेद्रियग्रहणाच्च पंचभिरिन्द्रियैर्गृहयते द्रव्यं न रसादयः ; आश्रयत्वाच्च, द्रव्यमाश्रिता रसादयो भवन्ति, आरम्भ सामर्थ्याच्च द्रव्याश्रित आरंभो यथा विदारिगंधादि माहृत्य संक्षुघ विपचेदिन्येवमादिषु न रसादिषु आरम्भः , शास्त्रप्रमाणाच्च , शास्त्रे हि द्रव्यं प्रधान मुपदेशे हि योगानां यथा मातुलुंगान्गिमन्थौचेत्यादौ न रसादय उपदिश्यन्ते; कमापेक्षितत्वाच्च रसादीनां, रसादयो हि द्रव्यकमपेक्षन्त, यथा तरुणे तरुणाः संपूर्णे संपूर्णा इति; एकदेशसाध्यत्वाच्च; द्रव्याणमेकदेशानामपि व्याधयः साध्यन्ते, यथा महावृक्षक्षीरेणेति; तस्माद द्रव्यं प्रधानं न रसादयः , कस्मान्निरवयवत्वात् द्रव्यलक्षणं तु 'कियागुण्वत् समवायिकारण' इति ।। सु.सू 40 .3

Dravya as a whole is more important to be remembered , the pharmacological properties described as Rasa, Guna , Veerya, vipaka etc. only qualify the activity and can never represent the drug individually. The message of Acharyas clearly emphasizes that the drug [dravya] has to be understood and remembered by its pharmacological utility as described in the texts [after long experience] and need not be speculated just based on individual properties;

The importance is assigned to the dravya due to the following reasons:

1. Because of its organized nature. . व्यवस्थितत्वात्

For instance, it is the physical nature of the drug material that exists as an organized [gross] entity and not the [other four – rasa etc.,]

For example a fruit which is the drug material, viz., the organized stuff [or , the crude drug in its modern parlance]. Here the rasas etc., that reside in an unripe, young fruit do not exist [i.e., they change] when the latter ripens [but the fruit itself exists as organised as before. We are not wrong therefore to assert that it is the crude drug that is of greater value than its rasas, etc., which go on changing.

2. Because of its invariability- नित्यत्वात च.

Take the example of any category of [a prepared drug material like] kalka etc. a given kalka may have its rasa and odor propitious or deranged [but these variations in property will not enable us to consider that the kalka itself is nolonger there i.e., we cannot ignore the existence of the kalka itself giving undue importance to its rasa ,odor etc., viz., the former nature of the drug i.e., its crude drugness is fixed and constant.

3. Because (any) specific category (of the concerned) is setteled and does not change (i.e., in its physical nature itself) – स्वजात्यवस्थात च.

For instance, a (drug) mateiral that is parthiva(now the earth) will not attain any other state (of apa, taijasa, etc.,) later. This holds good for (drug materials of) all the four ~~8520~~/other categories of (ap, taijasa etc.,)

3. Because it can be grasped by all the five senses (that we have)
पंचेंन्द्रिय ग्रहणत्वात च.

It is the physical nature or the drug material that can be felt by all of our five senses and not the individual taste etc., (where in it is only one or two senses and not the all five).

4. Because, it acts as the substratum *आयश्रयत्वात च.*

(for all the other four properties or, in other words) the taste, etc., occur with in the framework of the crude drug material (and not the other way)

5. Because all therapeutic activities (i.e., the activities of physicians) commence with this drug material. *आरंभ सामर्थ्यात च.*

It is this that forms the standpoint for all medicative procedures. For instance, there can be a directive (in a medical text) 'you should first collect the crude drug material like vidari gandha etc., Powder it and then cook it etc., (but) you cannot start (giving directives in this way) for the taste and the like (i.e., you cannot say " you should first collect a madhura rasa, cook it and so on).

6. Because many a technical work so declares. *शास्त्र प्रमाणाच्च .*

In (making the) combination drug, it is the crude drug such as matulunga, agnimantha, etc., that are mentioned (as the) ingredients (concerned) and not the taste etc., (i.e., there can be a directive that in preparing any combination drug, you take matulunga, agnimantha etc., and not the madhura rasa and ushna virya and so on)

7. Rasa and the like go on appearing gradually only,[*क्रमपेक्षितत्वात च*] i.e., one after the other (unlike the crude drug material which exists as such from the beginning itself).

8. Because many (different pharmacologically active) substances are situated in a single place with a crude drug. *एकदेशसाध्यत्वात् च .*

in a variety of substances (in this way) with the (single) crude drug, as for etc., in the milky fluid of the mahavriksha (which is known to contain a variety of medicinally useful substances). It is because of all these reasons, (these experts say) that it is the crude drug material (i.e., DRAVYA) that is of greater value than the other four of the above.

9 तरतमयोगानुपलब्धि - तरतमयोग अनुपलब्धि । र.वै.सूत्र 1-100. तरतमयोगो रसादिपु दृष्टः । मधुरो मधुरतरः मधुरतमः शीतरः शीततमः ..... इति । द्रव्येषुनास्ति यष्टिमधुकरो यष्टिमधुकतम इति । तस्मात द्रव्यं प्रधानं इति ।

Tartamyog is seen in the case of rasas. For example , there are states like sweet, sweeter, and sweetest. In gunas also there are states like heavy, heavier and heaviest. This is so, because the gunas present in the dravyas are variable. This is not applicable to karya dravyas . since the karya dravyas are stable and fixed, there is no need to ascertain various dravyas of comparision between various states of the sme dravya and thereby produce instability. We do not feel the need of saying goldier or goldiest like heavier and heaviest since dravyas like gold are stable and non changing. This also rates dravya as speriour. Degrees of comparision are not applied to dravya.

11.*विकल्पसामर्थ्य र.वै. 1-105*

..... विविधः कल्पो विकल्पः कल्ककषायादिभेदेन तस्मिन् विकल्पे सामर्थ्यात् तत् सर्व सामर्थ्यात् तत् सर्व द्रव्यस्य एव न अन्यस्य इति ।

"Vikalpa meanes various kalp" . for example powder, decoction, juice, siddha ghrita, asava, arista, etc., only dravyas can be used in these various forms. There is no such possibility in case of rasa, guna etc., this is also a chief reason for establishing te superiority of dravyas. A decoction of rasas and gunas is not possible.

12. *प्रतिघातसामर्थ्यातत् र.वै.1-106*

प्रतिघात आवरणं तस्मिन सामर्थ्ये द्रव्यस्यैव भवति मूर्तिमत्वात ।

Dravya has a definite form. Two dravyas cannot simultaneously occupy the same space. This called " Pratighata Samathya" of dravyas. This is not so in the case of gunas. Each guna does not need a separate space for its existence. The part of dravya which sustains the rasa can also sustain " gandha (smell) at the same time. Every minute particle of a dravya occupies a separate space.

The emphasis laid by acharyas on the crude drug material clearly implicates the **quality control attitude** of Acharyas. The conclucing remarks of Susrutha listed below clearly depict the same spirit:

पाकोनास्ति विना वीयात् वीर्यं नास्ति विना रसात ।।
रसो नास्ति विना द्रव्याद् द्रव्यं श्रेष्ठतमं स्मृतम् ।। सु.सू 40. 15

जन्म तु द्रव्यरसयोरन्योऽन्यापेक्षिकं स्मृतम ।।
अन्यो न्यापेक्षिकं जन्म यथा स्यात देहदेहिनो : ।। सु.सू 40. 16
वीर्यसंज्ञा गुणा येष्टौ ते ति द्रव्याश्रयाः स्मृताः ।।
रसेषु न भवन्त्येते निर्गुणास्तु गुणाः स्मृताः ।। सु.सू 40. 17

द्रव्ये द्रव्याणि यस्माध्दि विपच्यन्ते न पङ्रसाः ।।
श्रेष्ठं द्रव्यमतो ज्ञेयं शेषा भावास्तदाश्रयाः ।। सु.सू 40. 18

There can be no vipak with out veerya, veerya cannot exist without rasa and rasa has no existance devoid of dravya; hence dravya is superior amoungst all. The gunas and veerya reside in the dravya and in gunas. Gunas etc., are only dependent variable and it is the dravya which acts as the repository of all these things attains more importance .

By assigning the great importance to the crude drug i.e., dravya they are clearly sending the message that unless the material stated is **procured in the prescribed form** only , it will deliver any therapeutic action. **The message is drug standardization begins at procuring right raw material and the rest like processing, therapeutic utilization etc., will automatically follow.**

C. The basic qualities described for an ideal drug are:

बहुकल्पं बहुगुणं सम्पन्नं योग्यमौषधम् ।अ.सं सू.2.23

A drug must have multi dimensional therapeutic activity[बहुगुण] , many dosage forms[बहुकल्प] so that it can be the ability to deliver desired action, and safe enough not to induce/produce complications after its use[3].

D. Importance of Natural characters, Place of collection, suitable storage and safety:

प्रशस्त देशे संजातं प्रशस्तेऽहनि चोधृतम् । भा.प्र.भाग 1.91
अल्पमात्रं बहुगुणं गंधवर्ण समन्वितम् ।।

दोषघ्नंमग्लानिकरमधिकं न विकारियत् ।
समीच्य काले दत्तं च भेषजं स्यात् गुणावहम् । ।भा.प्र.भाग1.175; 176

Any herb should be collected from its natural habitat, which is free from contamination, pollution, must have proper color, taste, smell, and other characteristics of that particular herb. They should be stored properly so that their potency is retained. For this they must be collected from a pollution free place and stored in a tidy place free from any infestation. Which damage the quality and efficacy of the drug and more over may produce hazardous effects[4].

E. Importance of Knowing the Sanskrit and Vernacular nomenclature and the resources to obtain the information:

(stepping stone of Drug standardization – identification)

औषधं ह्य नभिज्ञानं नामरूप गुणैस्त्रिभिः ।
विज्ञानं चापि दर्युक्तमनर्थायोपपघते । ।च.सू-126

Charaka clearly emphasizes the need to know characteristic features of a drug Viz., names, appearance(cardinal features) , qualities (includes physical chemical and biological activities) . failure to do so is accountable to incompetence of the physician[5].

एकंतु नाम प्रथितं बहूनामेकस्य नामानि तथा बहूनि ।
द्रव्यस्य जात्याकृतिवर्ण वीर्य रस प्रभावादि गुणैर्भवन्ति । ।

नामश्रुतं केनचिदेकमेव तेनैव जानाति स भेषजंतु ।
अन्यस्तथान्येनतु वेत्ति नाम्ना तदेव चान्योऽथ परेण कश्चित । ।

बहून्यतः प्राकृतसंस्कृतानि नामानि विज्ञाय बहूँश्च पृष्ट्वा ।
दृष्ट्वा च संस्पृश्य च जतिलिंगैः विद्याद् भिषग् भेषज मादरेण । ।
Adarsh Nighantu

गेपालस्तापसा व्याधा ये चान्ये वनचारिणः ।
मूलजातिश्च ये तेभ्यो भेषज व्यक्तिरिश्यते । ।

प्रयोजनाः संति वनचरास्ते गोपादयः प्राकृतनाम संज्ञाः ।
प्रयोजनार्थं वचनप्रकृतिर्यस्मित्यादेशः ।च. सू.

The herbs or any drug of importance is widely used by the people in various places and call it with different names. Just knowing the sanskrit names as described in the Nighantu and sources is of not much use to collect any drug as it will have a different name at that particular place. Hence it is expected from a physician that he makes sure to learn as many vernacular (prakrit) names as possible along with knowing the sanskrit names.

The best source of knowing the botanical identity therapeutic uses and vernacular name is the tribes, and the cattle grazers who have extensive field experience in identifying and using the herbs available in the forests (**Ethnobotany**). In the present scenario knowing the scientific nomenclature and learning the botanical identification will help to solve the problem of identification which is the stepping stone of Drug standardization[6].

Next step in drug identification is based on the morphological and other important characteristic features; an attempt has been made by ayurvedic acharyas and much earlier by rishis in Vedas to classify the drugs based on morphology etc. which can be considered as a mile stone in Drug standardization temarament prevalent at that time; some examples of such classification are :

F. Morphological considerations of plants from Vedic and Ayurvedic literature

1. प्रसृणवन्ती स्तम्बिनीरेकशुंगा प्रतन्वतीरोषधीरावदामि ।
अंशुमती कंडिनीर्या विशाखाव्हयामि ते वीरुधो विश्वदेवी
रुग्या पुष्पमजीवनी ।। शौ. 8.25

2.पुष्पमती प्रसूमती फलिनीतफला उत ।
स मातर इव दुहामस्ता अरिष्टाजायते । शौ. 8.26

3.अवकाल्वो उदकात्मान ओषधयः ।
व्युपन्तु दुरितं तीक्ष्णश्रृंग्यः ।। शौ. 8.27

These mantra from atharvaveda clearly speaks about morphological characters of herbs as; prastrunavanti- the spreading herbs, phalavati- ripe with fruits, sthambini-bushy herbs, ekashunga- the one buded, prtanavanti spreading, amshumati- glowing, dandini, vishakha- with out branches, etc.

मूलत्वक्सारनिर्यासनालस्वरसपल्लवाः ।
क्षाराः क्षीरं फलं पुष्पं भस्म तैलानि कण्टकाः ।
पत्राणि शुंगाः कन्दाश्च प्ररोहाश्चोद्भिदो गणाः ।च.सू.1

Charaka clearly describes the morphology or the plants as : Mula-root, twak- bark, niryasa- exudate, shung- leaf buds, kand-tuberous roots, praroha- tender tips, kshara-the alkali residue of plants, bhasma- ash, tail- oil etc.

.....औद्भिदंतु चतुर्विधम ।
वनस्पतिः तथा वीरुध् वानस्पत्यः तथा ओषधिः ।।
फलैः वनस्पतिः पुष्पैः वानस्पत्यः फलैः अपि ।
ओषध्यः फलपाकान्ताः प्रतानैः
वीरुधः स्मृतः ।।च.सू-1

a. Vanaspati –The trees which have very minute flowers and hence cannot be obviously seen are called vanaspati- apushpa, antahpushpa, or guhyapushpa (गुह्यपुष्प फलिनः)

b. Virudh: Plants growing as creepers or growing in clumps or clusters are called virudh e.g., guduchi . patol, karavellak - प्रतानैर्वीरुधः स्मृतः - प्रतानशब्देनन लता गुल्मश्च गृह्यते । च. सू. 1.70 टीका.

c. Vanaspatya- the plants which bear visible flowers and then bear ripen are called vansptya e.g. , like mango

d. Oshadhi – the plants which dry out once the fruits ripen and are dcaled oshadhi e.g., wheat, rice, barley etx.

G. Guidelines for collecting useful part from a selected plant source for medical use:

Acharyas have clear understanding of importance of proper identification and the useful part of every plant, they have mentioned in their texts. The carefully documented literature clearly depicts the spirit of appling quality control methods for drug collection as mentioned in

bhavaprakash nighantu purvardha. The examples quoted below summerise the importance of collecting the suitable plant part according to the nature of the drug . the details are as follows;

अतिस्थूलजटा याः स्युस्तासां ग्राह्यास्त्वचो ध्रुवम् ।
गृह्णीयातत्सूक्ष्ममूलानि सकलान्यपि बुद्धिमान् ।।
भा. प्र. प्र.खं 5.111

महान्ति येषां मूलानि काष्ठगर्भाणि सर्वतः ।
तेषां तु वल्कलं ग्राह्यं ह्रस्वमूलात्रिन सर्वशः ।।
भा. प्र. प्र.खं 5.112

न्यग्रोधादेस्त्वचो ग्राह : सारः स्याद्बीजकादितः ।
तालीसादेश्च पत्राणि फलं स्यात त्रिफलादितः ।।
भा. प्र. प्र.खं 5.113

क्वचिन्मूलं क्वचित्कन्दः क्वचित पत्रं क्वचित्फलम ।
क्वचित्पुष्पं क्वचित्सर्वं क्वचित्सारः क्वचित्वचः ।।
भा. प्र. प्र.खं 5.114

चित्रकं सूरणं निम्बो वासा च त्रिफला क्रमात ।
धातकी कण्टकारीच खदिरः क्षीरपादपः ।।
भा. प्र. प्र.खं 5.115

Bark has to be procured from bigger trees; Small plants have to be collected as whole; Vata, Aswattha etc., Bark is useful part; Vijaysar, Chandan- Sar(pith); Taleesa- Patra; Haritaki, Amalaki, Vibhitaki- Fruits; Chitrak- Mulam; Suran- Tubour; Nimba, Vasa- Patra(leaves), Dhataki- flowers; Kantakari- Panchang; Khadira- Sar (pith);

क्वचिन्निम्बस्य गृह्णीयातत्पत्राभावे त्वचमपि ।
बालं फलं तु बिल्वस्य पक्वमारग्वधस्य च ।।
भा. प्र. प्र.खं 5.116

अन्ये नुक्ते जटा ग्रह्या भागेऽनुक्ते खिलं समम् ।
पात्रेऽनुक्ते मृदः पात्रं कालेनुक्ते स्वहर्मुखम् ।। भा. प्र. प्र. खं 5.117

- In place of Nimbapatra Bark is acceptable;
- Bilva phalamajja (fruit pulp)should be unripe for medical usage;
- Aragwadh (Fruit pulp) –Should be collected in ripen state;

- If any specific part is not described in literature of Bhaishajya Kalpana Mula-Root has to be taken;
- Any specific ratio is not mentioned- it is deemed to take equal parts;
- In the absence of any specific vessel mud vessel has to be taken;
- In the absence of specific time – morning hours is suitable time;

नावन्येव हि योज्यानि अन्याण्ययखिलकर्मसु ।
विना विडंगकृष्णभ्यां गुडधान्यमाक्षिकैः ।।
भा. प्र. प्र.खं 5.118

- Except for Vidang, Pippali, Gud (Jaggery), Godhum, Dhanyak, Ghrita and Madhu rest of all products can be used when they are freash [Vidang etc., older the better].

पुराणंतु प्रशस्तं स्यात्ताम्बूलं कान्जिकं तथा ।
शुष्कं नवीनद्रव्यं तु योज्यं सकलकर्मसु ।। भा. प्र. प्र.खं 5.119

आर्द्रं तु द्विगुणं युन्ज्यादेवं सर्वत्र निश्चयः ।
गुडूची कुटजो वासा कूष्मांडश्च शतावरी ।।
भा. प्र. प्र.खं 5.120

अश्वगन्धा सहचरः शतपुष्पा प्रसारणी ।
प्रयोक्तव्याः सदैवार्द्रा द्विगुणं नैव कारयेत ।।
भा. प्र. प्र.खं 5.121

वासानिम्बपटोलकेतकबलाकूष्माण्डकेन्दीवरवर्षाभूकुटजाश्च
कन्दसहिता सा पूतिगन्धामृता ।
एन्द्रीनागबलाकुरुण्टकमुरच्छत्रामृताः सर्वदा सार्द्रा एव तु न
क्वचिद् द्विगुणिताः कार्येषु योज्या बुधैः ।।
भा. प्र. प्र.खं 5.122

- Tambul (Beatle leaves), Kanji, older the better;
- In the absence of dry material , use freash drug in double quantity [exceptions: Guduchi, Kutaj, Vasa, Kaushmand, Shatavari, Aswagandha, Sahachar, Shatapuspi, Prasarini etc.,]

*घृतं तैलं च पानीयं कषायं व्यंजनादिकम् ।
पक्त्वा शीतकृतं चोष्णं तत्सर्वं स्याद्विषोपमम् ।। भा. प्र. प्र. खं 5.123

Ghrita, Tail, water, kwath (decoction) etc., once boiled and cooled must never be re warmed; if done so they act as poisons;

H. Dravyapareeksha

Qualities ascribed to identify the best drug for avoiding contamination and adulteration are duly recognized . Literature related to such information from Bhavprakash is mentioned here .

सूक्ष्मस्थिमांसला पथ्या सर्वकर्मणि पूजिता ।
क्षिप्ताम्भसि निमज्जेद्या भल्लातक्यस्तथोत्तमः ।।
भा. प्र. प्र.खं 5.124

- Harieetaki- which has small seeds and thick seed coat is considered best for clinical usage;
- Bhallatak- which sinks in water;

वराहमूर्द्धवत्कन्दो वराहीकन्दसंज्ञकः ।
सौवर्चं तु काचाभं सैन्धवं स्फटिकप्रभम् ।।
भा. प्र. प्र.खं 5.125

- Surankand- Which has thick hairy surface and shape of Pig's face;
- Souvarch lavan- the sample which looks like dark glass;
- Saindhav lavan- which has crystal like appearance;

सुवर्णच्छविकं ज्ञेयं स्वर्णमाक्षिकमुत्तमम् ।
इन्द्रमुषप्रतीकाशं मनोहा चोत्तमा मता ।।
भा. प्र. प्र.खं 5.126

- Swarnamakshik- Lusture like that of gold;
- Manashila- which has colours of Rainbow;

श्रेष्ठं शिलाजतु ज्ञेयं प्रक्षिप्तं न विशीर्यते ।
तोयपूर्णे कांस्यपात्रे प्रतानेन विवर्धते ।। भा. प्र. प्र.खं 5.127

- Shilajit- the sample which does not disperse but spreads like threads on the surface of water placed in a bronze [kamsya] vessel.

कर्पूरस्तुवरः स्निग्धा एला सूक्ष्मफला वरा ।
श्वेतचन्दनमत्यन्तं सुगन्धि गुरुपूजितम् ।।
भा. प्र. प्र.खं 5.128

- Karpur- which is snigdha;
- Ela- which has small fruits;
- Shwet chandan- which has good odour, and heavy;

रक्तचंदनमत्यन्तं लोहितं प्रवरं मतम् ।
काकतुण्डनिभः स्निग्धो गुरुः श्रेष्ठो गुरुर्मतः ।।
भा. प्र. प्र.खं 5.129

- Rakta Chanda – which red [lohita] in colour and heavy;
- Agaru- Which has dark black colour as that of a crow's beak is considered best;

सुगन्धि लघु रुक्षं च सुरदारु वरं मतम् ।
सरलं स्निग्धमत्यर्थं सुगन्धि च गुणावहम् ।।
भा. प्र. प्र.खं 5.130

- Devdaru- Sugandhi[has good odour], and laghu is best;
- Saral- Shigdh and sugandh yukta.

अतिपीता प्रशस्ता तु ज्ञेया दारुनिशा बुधैः ।
जातीफलं गुरु स्निग्धं समं शुभ्रान्तरं वरम् ।।
भा. प्र. प्र.खं 5.131

- Daru Haridra- Which has deep yellow colour;
- Jatiphala- which is heavy, unctous [snigdha] and looks white from inside;
- Mridwika- which has the shape of Nipple of a cow;

मृद्वीका सोत्तमा ज्ञेया या स्याद् गोस्तनसन्निभा ।
करमर्दफलाकारा मध्यमा सा प्रकीर्तिता ।। भा. प्र. प्र.खं 5.132

खण्डं तु विमलं श्रेष्ठं चन्द्रकान्तसमप्रभम् ।
गव्याज्यसदृशं रुच्यं गन्धं मधु वरं मतम् ।।
भा. प्र. प्र.खं 5.123

- Khand sharkar- crustal white in colour and has the taste of Madhu and ghrita;

I. Nomenclature applicable to some dravya in Bhaishajya kalpana literature [भेषजसंग्रह संकेतः]

Ayurvedic literature is full of Formulations and single drug uses for managing various ailments . At times the author may not specify every ingredient in detail used in the kalpa and merily use a symbolic term in its place. For solving this problem we find many efforts by various authors like Sharangadhara, Bhavamishra who have tried to specify the terminolgy used in the kalpas. This is one of the very important standardization aspects which is thoroughly recorded and serves very well even today. Few examples from Bhavprakash Purvakhanda reveal these details :

लवणं सैंधवं प्रोक्तं चन्दनं रक्तचंदतम् ।
चूर्णलेहासवस्नेहाः साध्या धवलचन्दनैः ।।
कषायलेपयोः प्रायो युज्यते रक्तचन्दनम् । भा. प्र. प्र.खं 5.128

अन्तः सम्मार्जने ज्ञेया ह्राजमोदा यवानिका ।
बहिः सम्मार्जने सैव विज्ञातव्याऽजमोदिका ।। भा. प्र. प्र.खं 5.129

पयः सर्पिः प्रलेपेषु गव्यमेव हि गृह्यते ।
शकृद्रसो गोमलको मूत्रं गोमूत्रमुच्यते ।। भा. प्र. प्र.खं 5.130

Lavan always means – Saindhav Lavan

Chandan- 1. For preparation of Asava, Avleh, Sneha Etc, Swet chandan is to be taken;
2. For preparation of Kashayas and for regular use – Raktachandan has to be taken.

Ajmoda 1. For internal use- Yavani
2. For external use- Ajmoda .

Milk, Ghrita, Goras [dung], Urine etc - only cow's products have to be used if not otherwise mentioned;

Apart from this in sharangadhara samhita we find thorough mentioning of Dravya mana [mesurements] for liquids , Solids and time which are relevent even in present time also. They are very well serving the purpose of quality control and are the basic guidelines for manufacturing Ayurvedic formulations.

J. Concept of drug substitution [प्रतिनिधि द्रव्य]

In medical practice it is not always easy to procure the recommended drugs as per the texts. Some time due to unavailability of the drug it becomes mandatory to substitute it with a suitable and equally potent drug so that the desired therapeutic effect is achieved.

By extensively mentioning the various substitutions the later texts like Bhavaprakash have enriched the pharmacopoea of ayurveda. They have also proved the efficacy of careful observation of clinical properties of various drugs the similarities and dissimilarities between them which has enabled them to document the pratinidhi dravyas.

Important use of the pratinidhi dravyas is, even a drug is not available due to extinction, improper identification and other reasons we will still have an alternative source, which will solve the problem; some guideline for Drug substitution are as follows;

General guide lines for substitution:

Drugs which have similar properties and actions can be substitutes with each other;
The Pradhana Dravya which is specifically aimed curing a disease should not be substituted unless other wise clearly stated, and replacement is possible only with equally important drug.

List of Pratinidhi dravyas described in bhavprakash;

S.no	Name of the Dravya	Substitute
1.	Chitrak [Plumbago zeylanica Linn.]	Apamarg Kshar [Achyranthus Aspera Linn.,]/ Danti Mula [Baliospermum montanum Muell.]
2.	Dhanvayas [Alghahi camelorum fisch.]	Duralabha [Fagonia arabica Linn.]
3.	Tagar [Valeriana wallachi DC]	Kustha [Saussurea lappa C.B.Clark.]
4.	Avartani [Helicterus isora Linn]	Jingani Twak [Odina woodier roxb]
5.	Himsra	Mankanda [Alocasia indica (Roxb) Schott.]
6.	Lkshmana [Ipomea sepiaria Koen]	Mayurshikha [adiantum caudatum Linn]
7.	Bakul [Mimosops elangi]	Kamal [Nelumbium speciosum Willd]
8.	Nila Kamal	Kumud
9.	Jatiphala [Myristica fragrance Houtt.]	Lavang [Syzizium aromaticum]
10.	Arka [Calotropis.,], Snuhi Dugdha	Juice of Leaves
11.	Pushkarmul [Inula racemosa], Langali [	Kustha – Saussaria lappa / Costus speciosus]

	Gloriosa superba]	
12.	Pippalimul [Piper longum]	Chavya [
13.	Bakuchi [Psoralia corylifolia Linn.]	Prapunnat –Chakramard·[Cassia tora Linn]
14.	Daruharidra [Berberis aristata DC]	Haridra [Curcuma longa Linn]
15.	Rasanjan [antimony]	Daruharidra Ghan
16.	Sourastri	Turuti [Alum]
17.	Bharangi [Clerodendron serratum spreng]	Talees patra [Abies webbiana / Kantakari mul [Solanum surettense]
18.	Yastimadhu [Glycyrrhiza glabra Linn]	Dhataki [Woodfordia fruiticos Kurz]
19.	Amlavetas [Garcinia pedunculata Roxb]	Chukra [Rumex vesicarious Linn]
20	Draksha	Kashmari phala/ Banduk
21	Nakha	Lavanga
22.	Kasturi [Musk]	Kankol [Piper cubeba Linn.F]
23.	Kankol [Piper cubeba Linn.F]	Jatipushpa { jasmin flowers]
24.	Karpur [Camphor]	Sugandhi Musta/ Grandhiparna [Polygonum aviculare Linn.]
25.	Kumkum [Crocus sativus Linn.]	Kusumbha [Carathmus tinctorius Linn- bastard saffron]
26.	Sweth chandan [santalum album Linn]	Karpur / Rakta chandan [Pterocarpus santalinus Linn f.]
27.	Rakta chandan [Pterocarpus santalinus Linn f.]	Ushir [vetivaria zizanoides (Linn) Nash.]
28.	Ativisha [Aconitum heterophyllum Wall]	Musta [Cyperus rotundus Linn]
29.	Haritaki [Terminalia chebula Retz.]	Amalaki [Emblica officinalis Gartn.,]
30.	Nagkeshar [Mesua ferrea Linn]	Kamal keshar
31	Meda/ Mahameda	Shatavari [Asparagus racemosus Willd]
32.	Jivaka / Rishabhak	Vidarikanda [Ipomea digitata Linn/ Puraria tuberosa]
33.	Kakoli/Ksheera Kakoli	Aswagandha [withania aswagandha Kaul]
34.	Riddhi/ Vriddhi	Varahi Kand [Diascorea bulbifera Linn]
35.	Varahi Kanda	Charmakaluk
36.	Bhallatak intolerance	Rakta chandan
37.	Bhallatak	Chitrak
38.	Ikshu	Nal
39.	Swarna	Swarna makshik
40.	Rajat	Rajat Makshik
41.	Swarna makshik	Swarna Gairik
42.	Swarna bhasma/ Rajat Bhasma	Kantaloh Bhasma
43.	Kanta loh Bhasma	Teekshna loh Bhasma
44.	Mukta [pearls]	Mukta shukti [Shell]
45.	Madhu	Old Jaggery
46.	Milk	Mudga/ Masoor Ras.

चित्रका भावतो दन्ती क्षारः शिखरिजोऽथवा ।
अभावे धन्वयासस्य प्रक्षेप्या तु दुरालभा । भा. प्र. प्र.खं 5.151

तगरस्याप्यभावे तु कुष्ठं दद्यादभिग्वरः ।
मूर्वाभावे त्वचो ग्राह्या जिन्जिनी प्रभवा बुधैः ।।
भा. प्र. प्र.खं 5.152

अहिंसाया अभावेतु मानकन्दः प्रकीर्तितः ।
लक्ष्मणाया अभावेतु नीलकण्ठशिखा मता ।।
भा. प्र. प्र.खं 5.153

वकुलाभावतो देयं क ारोत्पल पंकजम् ।
नीलोत्पलस्याभावेत तुं कुमुदं देयमिष्यते ।।
भा. प्र. प्र.खं 5.154

जातीपुष्पं न यत्रास्ति लवंगं तत्र दीयते ।
अर्कपर्णादिपयसो ह्यभावे तदसो मतः ।। भा. प्र. प्र.खं 5.155

पौष्कराभावतः कुष्ठं तथा लाग्गल्याभावतः ।
स्थौणेयकस्यभावे तु भिषग्भिर्दीयते गदः ।।
भा. प्र. प्र.खं 5.56

चविकागजपिप्पल्यौ पिप्पली मूलवत्स्मृतौ ।
अभावे सोमराज्यिास्तु प्रपुन्नटफलं मतम् ।।
यदि न स्याद् दारुनिशा तदा देया निशा बुधैः ।
भा. प्र. प्र.खं 5.157

रसाञ्जनाभावे तु सम्यग्दार्वी प्रयुज्यते ।
सौराष्ट्र ाभावतो देया स्फटिका तद्गुणा जनैः ।।
भा. प्र. प्र.खं 5. 158

तालीसपवकाभावे स्वर्णताली प्रशस्यते ।
भाग्र्याभावे तु तालीसं कण्कारीजटाऽथवा ।।
भा. प्र. प्र.खं 5.159

रुचकाऽभावतो दघाल्लवंगं पांशुपूर्वकम् ।
अभावे मधुयष्टास्तु धातकीं च प्रयोजयेत ।। भा. प्र. प्र.खं 5.160

अम्लवेतसकाभावे चुक्रं दातव्यमिष्यते ।
दाक्षा यदि न लभ्येत प्रदेयं काश्मरीफलम् ।।
भा. प्र. प्र.खं 5.161

तयोरभावे कंकोलं क्षेपणीयं विदुर्बुधः ।
लवङ्गकुसुमं देयं नखस्याभावतः पुनः ।।
भा. प्र. प्र.खं 5.162

कस्तूर्याभावे कंकोलं क्षेपणीयं विदुर्बुधैः ।
कंकोलस्याभावे तु जातीपुष्पं प्रदीयते ।। भा. प्र. प्र.खं 5.163

सुगन्धिमुस्तकं देयं कर्पमराभावतो बुधैः ।
कर्पूराभावतो देयं ग्रन्थिपर्णं विषेषतः ।। भा. प्र. प्र.खं 5.164

कुंकुमाभावतो दघात्कुसुम्भकुसुमं नवम् ।
श्रीखण्डचन्दनाभावे नवोशीरं विदुर्बुधाः ।।

भा. प्र. प्र.खं 5.165

अभावेत्वेतयोर्वैद्यः प्रक्षिप्तदक्तचन्दतम् ।
रक्तचन्दनकाभावे शिवाभावे शिवा मता ।।
भा. प्र. प्र.खं 5.166

मुस्ता चातिविषाभावे नवोशीरं विदुर्बुधाः ।
अभावे नागपुष्पस्य पद्मकेशरमिष्यते ।। भा. प्र. प्र.खं 5.167

मेदाजीवककाकोली ऋद्धिद्वन्द्वेपि वा सति ।
वराहीविदार्यश्वगन्धावराहीश्च क्रमात् क्षिपेत् ।।
भा. प्र. प्र.खं 5.168

वराहाश्च तथाभावे चर्मकारालुको मतः ।
वाराहीकन्दसंज्ञस्तु पश्चिमे गृष्टिसंज्ञकः ।।
भा. प्र. प्र.खं 5.168

वाराहीकन्द ऽवान्यश्चमेकारालुको मतः ।
अनूपसम्भवे देशे वराह इव लोमवान ।। भा. प्र. प्र.खं 5.170

भल्लतकासहत्वे तु रक्तचन्दनमिष्यते ।
भल्लाताभावश्चित्रं नलश्चेक्षोरभावतः ।। भा. प्र. प्र.खं 5.171

सुवर्णाभावतः स्वर्णमाक्षिकं प्रक्षिपेद् बुधः ।
श्वेतं तु माक्षिकं ज्ञेयं बुधैः रजत वद् ।। भा. प्र. प्र.खं 5.172

माक्षिकस्याप्यभावेतु प्रदघात्स्वर्णगैरिकम् ।
सुवर्णमथवा रौप्यं मृतं यत्र न लभ्यते ।। भा. प्र. प्र.खं 5.173

तत्र कान्तेन क्रर्माणि भिषक्कुर्याद्विचक्षणः ।
कान्ताभावे तीक्ष्णलोहं योजसेद्वैद्यसत्तमः ।।
भा. प्र. प्र.खं 5.174

अभावे मौक्तिकस्यापि मुक्ताशुक्तिं प्रयोजेत् ।।
मधु यत्र न लभ्येत तत्र जीर्णगुडो मतः ।।
भा. प्र. प्र.खं 5.175

मत्स्यण्डाभावतो दधुर्भिषजः सितशर्कराम् ।
असम्भवे सितायास्तु बुधैः खण्डं प्रयुज्यते ।।
भा. प्र. प्र.खं 5.176

क्षीराभावे रसो मौद्गोमासुरो वा प्रदीयते ।
अत्र प्रक्तानि वस्तूनि यानि तेषु च नेषु च ।।
भा. प्र. प्र.खं 5.177

योज्यमेकतराभावे पतं वेद्येन जानता ।
रसवीर्यविपाकाद्यैः समं द्रव्यं विचिन्त्य च ।।

भा. प्र. प्र.खं 5.178

युंज्याद्विविधमन्यश्च द्रव्याण्प तु रसादिवित् ।
योगे यदप्रधानं स्यात्तस्य प्रतिनिधिर्मतः ।।
भा. प्र. प्र.खं 5.179

यत्तु प्रधनं तस्यापि सदृशं नैव गृहाते ।
व्याधेरयुक्तं यद् द्रव्यं गणोक्तमपि तत्त्यजेत् ।
अनुक्तमपि युक्तं यघोजयेत्तद्रसादिकवित् ।।
भा. प्र. प्र.खं 5.180

K. Concepts of Shelf life from Ayurveda:

The next step in drug standardization is correct manufacturing procedures and storage which will ensure the shelf life and safety of its consumption ayurvedic Pharmaceutical s the bhaishajya Kalpana very eloberately discusses these principles and advocates Good Manufacturing practices[GMP] to ensure the quality of the drug Following are some details about shelf life and its importance:

गुणहीनं भवेद्वर्षादूध्वं तद्रूपमौषधम् ।शा.प्र.खं. 3.54
मासा,यात्तथा चूर्णं हीनवीर्यतव माप्नुयात् ।
हीनत्वं गुटिकालेहो लभेते वत्सरात्परम् ।।शा.प्र.खं. 3.55
हीनाः स्युर्घृत तैलाघा धातुर्मासाधिकास्तथा ।
ओषध्यो लघुपाकाः स्युर्निर्वीर्यो वस्तरात्परम् ।।शा.प्र.खं. 3.56
पुराणः स्युर्गुणेयुक्ता आसवा धातवो रसा।

1. All the drugs tend to loose their potency at the end of one year after collection (if not stored Properly)
2. Churna will have a shelf life of – 2 months
3. Guti, Vati- 1 year
4. Avaleha, Ghrita, and Taila – 16 months,
5. Annuals (ओषध्याः फलपाकान्ताः .ओषधि) loose their potency after one year.
6. Asava/Arista, Dhatu, upadhatu, bhasmas etc retain their therapeutic effect through their life (generally do not loose the therapeutic effect even on long run) [5].

Various aspects discussed here are only a reflection of vast amount of codefide literature [related to dravyaguna and bhaishajya kalpana] available in the form of Samhitas and other Ayurvedic literature. It of great advantage for a ayurvedic practioner to have such an amount of enormous amount of intellectual wealth [inspite of the heavy losses incured due to various historical reasons] to enrich the conceptual and practical knowledge which no doubt puts him on par with the current scintific community abd methodology .

This is the high time for every ayurvedic learner to shead the useless weight of low self esteem and to rise to the expectation and standards set by great Seers who have conceived this great Science which will surely bring the uniformity of understanding and practice which is the true purpose of Standardization.

References:

1. Charaka Samhita with Ayurveda Deipika commentary, Chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint,1992.
2. Acharya Shri Radhakrishna Parashar, Sharangadhara Samhita,with Hindi trans., Baidyanath Ayurved Bhavan, 1984.p. 12-13.
3. Ravi Dutta Tripathi, Astanga Sangraha- Sutra Sthan.3rd Ed., Chaukhamba Sanskrit Pratisthan, New Delhi, 1993.
4. Bhavamisra, Bhavaprakasha (first part), Kashi Sanskrit series 130, 8th ed. Chaukhambha Sanskrit sansthan, PO Box no. 1139, K37/116, Gopal Mandir lane Varanasi, 1993.
5. Charaka Samhita with Ayurveda Deipika commentary, Chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint,1992
6. Vd. Bapalal, Adarsha Nighantu, Chaukhambha bharati Academy II ed. 1968 (Reprint). part –I, P. 33
7. Acharya Shri Radhakrishna Parashar, Sharangadhara Samhita,with Hindi trans., Baidyanath Ayurved Bhavan, 1984.p. 12-13.
8. Susruta Samhita with Nibandha Sangraha commentary, chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint, 1994.
9. Shri. Pandit.Lalchandraji Vaidya., Bhavprakash- Pruvardha, Motilal Banarsidas Publication, N. Delhi 3rd Ed. Reprint 1986.
10. Vaidya V.M. Gogte., Ayurvedic Pharmacology and therapeutic uses of Medicinal plants., Bhavan's Book University., SPARC, Mumbai I english Ed., 2000.

Conceptual study

Part II

4. Conceptual Study Part -II

(Standardization of Botanicals)

Systematic Study of Crude Drugs

In the Indian Systems of Medicine (comprising of Ayurveda, Unani and Siddha), drugs of plant, animal and mineral origin, are used in their natural or so called 'Crude' forms singly or in their mixture or in combination, to make a compound preparation of formulation. Nearly 90 per cent of the Crude Drugs are obtained from the plant sources while about 10 per cent of the drugs are derived from animal and mineral sources.

The drugs of plant origin especially of herbaceous nature frequently used as whole plant; otherwise their parts such as Root, Stem, Leaf, Flower, Seed, Fruit and modifications of Stem and Root, Bark of a Stem or Root, Wood, and their Exudates or Gums etc. constitute single drugs in the Indian Systems of Medicine. These vegetable drugs are either used in dried form or some times as whole fresh or their juice.

The study of these crude drugs made with a view to recognize them is called Pharmacognosy (Pharmakon = Drug; Gignosco = to acquire knowledge of), meaning the knowledge or science of Drugs coined by Seydler in 1815.

In Pharmacognosy a complete and systematic study of a drug is done, which comprises of:

(i) Origin, common names, scientific nomenclature and family,
(ii) Geographical source (and history),
(iii) Cultivation, collection, preservation and storage,
(iv) Macroscopic, Microscopic and sensory (organoleptic) characters,
(v) Chemical composition wherever possible,
(vi) Identity, Purity, Strength and Assay,
(vii) Substitute and adulterants etc. such systematic study of a drug as complete as possible,

The basic chemical nature of cell-wall of almost all the plants is cellulosic. However, lignin, suberin, cutin or mucilage is deposited on the cellulose. Cellulose gives blue colour with chlorzinc-iodine solution or with cuoxam. (Copper- oxide-ammonia) reagent. Lignin present in the middle lamella and secondary cell-well of many vessels, fibers and sclerides gives red colour with phloroglucinol and concentrated hydrochloric acid. Suberin is present in cork and endodermis cells while cutin in the cuticle of leaf. Both are fatty in nature and when heated with Sudan Red-III give red colour.

Mucilage gives red colour with ruthenium red. The chemical constituents present in the drugs can be identified by chemical or micro chemical tests e.g.. Rhubarb rhizomes give red colour with 5% potassium hydroxide because of anthraquinone derivatives; strychnine present in Nux vomica gives purplish-red colour with ammonium vanadate and concentrated sulphuric acid.

TLC, PC and HPLC are now utilized in identification of drugs, their adulterant and their chemical constituents. Methods have been developed for quantitative estimation of the chemical constituents from Paper and Thin Layer Chromatography (TLC).

Morphological Or Organoleptic Evaluation

It refers to evaluation of drugs by colour, odour, taste, size, Shape and special features, like touch, texture, etc. it is a technique of qualitative evauation based on the study of morphological and sensory proviles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to impressions on organs of senses. The study of form of a crude drug is morphology while description of the form is morphograph. The fractured surfaces in cinchona, quillaia and cascara barks and quassia wood are important characteristics. Aromatic odour of umbelliferous fruits and sweet taste of liquorice are the examples of this type of evaluation. The ovoid tears of gum acacia, ribbon shaped characteristic of tragacanth, disc- shaped structure of nux-vomica, conical shape of aconite, quills of cinnamom etc. are important diagnostic characters. The general appearance of the lot of crude drug often indicates whether it is likely to comply with prescribed standards, such as percentage of seed in colocynth, stalk in clove, etc. overdrying, makes leaf drugs

and flowers brittle and cause them to break in transit making the task of morphological evaluation difficult.

The wavy shape of rauwolfia, pungent taste of capsicum and ginger, brown colour of cinnamom, odour and taste of spice-drugs like , asafoetida, black pepper, nutmeg, caraway, cummin, etc. are important diagnostic organoleptic characteristics[1].

Microscopical Methods of Examining Crude Vegetable Drugs

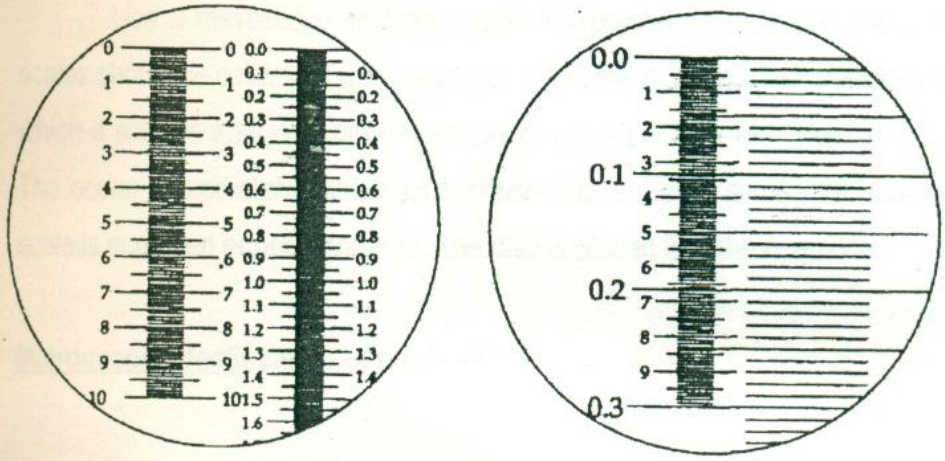
I. General Considerations

Calibration of the ocular micrometer

Place the ocular micrometer scale in the eyepiece of the microscope. Put the stage micrometer on the microscope stage and focus on the scale divisions. Both scales should be sharply defined in the field of view. Turn the eyepiece to place the scales in a parallel position and, if necessary, move the stage micrometer until the starting lines of both scales coincide. Now find another point, as far along the scale as possible, where two other divisions on the ocular micrometer and the corresponding length on the stage micrometer scale, in order to determine the length that is equivalent to one division on the ocular micrometer scale;

For example, if 100 divisions on the ocular micrometer scale are equal to 30 divisions on the stage micrometer scale, since the divisions on the stage micrometer scale are 0.01 mm apart, 100 ocular micrometer divisions are equivalent to 0.30 mm, therefore, each division on the ocular, micrometer scale represents 3.0μm. Since the calibrations apply only for a particular lens combination, it is advisable to combinations.

Fig. Alignment of the stage micrometer and the ocular micrometer



Method

Place the specimen on the microscope stage and focus on the object to be measured. Superimpose the ocular micrometer scale and read off the dimensions of the object. Multiply the number of scale divisions by the micrometer value to give the actual dimension in micrometers. By this method, using a 40 x objective and a 6 x eyepiece, measurements are corrected to the nearest 2μm, i.e. a dimension of 20μm is liable to an error of 2μm, or 10%, or ±5%; a dimension of 100 μm is liable to an error of 2% or ± 1%.

For curved and elongated objects, the measurement of lengths may be made using a microscopic equipped with a drawing apparatus or a camera Lucida. The instrument should be set up so that the image of the object, the drawing paper and the pencil are in focus simultaneously. With the stage micrometer in place, trace the lines of the stage micrometer scale upon the paper fastened on a drawing board. Tilt the drawing board, if necessary, until the divisions drawn upon the paper are equally spaced. The magnification is determined by measuring the distance between selected lines on the drawing paper and dividing by the distance between the corresponding lines on the stage micrometer. Place the specimen on

the microscope stage and trace the image object drawn on the paper and after straightening, it measure the length of the magnification to give the actual length of the object.

Measurement of specimen

Use a microscope with an ocular micrometer to measure the size of small objects. The scales should be calibrated using a stages micrometer, consisting of a glass slide of usual size, upon which a scale is engraved or photographed, usually 1 or 2 mm long, in 0.1 and 0.01 mm divisions. The ocular micrometer consists of a small disc of glass, across the diameter of which a 100-line scale is engraved or photographed. The disc is placed into the eyepiece.

Microscopic identification of crude -II

Methods of preparing specimens of crude materials of vegetable drugs for microscopic studies vary, depending on the morphological groups of drugs to be examined and also on the nature of the material i.e., entire, cut or powdered.

1. LEAVES, HERBS AND FLOWERS.

For examining leaves, herbs and flowers (entire or cut) under microscope, following methods are employed for clarification :

1A. ENTIRE AND CUT MATERIALS

(i) ***Entire materials*** — When examining entire leaves, herbs and flowers, take pieces of leaf (margin and vein of leaves only), herbs (only leaf) and flowers (only calyx and corolla) in test tube. Add a solution of caustic alkali or nitric acid to the test tube and boil for 1-2 minutes, pour the contents into a porcelain dish, drain off the liquid, wash the material with water and leave for sometimes. Remove the pieces of the material from the water with a spatula and put on the slide, add a few drops of the solution of *glycerol or chloral hydrate.* Crush the material with scalpel and cover with slip before examining.

(ii) ***Cut material*** — For examining cut leaves, herbs and flowers, take several pieces in a test tube and employ the same methods as described for entire materials.

Other methods employed for clarification of the material (leaf and stem) are described below: -

(a) **Leaf** - Boil pieces of leaves in a test tube with chloral hydrate for several minutes until completely clarified and then examine them in chloral hydrate solution. After clarification, leaf pieces are placed on a microscopic slide and then divided into two parts with the help of a scalpel or needle, and carefully turn one part. The leaf can be examined from both the surfaces i.e. dorsal and ventral.

(b) Stem - To examine stem material (without leaf) boil pieces in a solution of 10% *caustic a/kali* or in 40% *nitric acid.* Remove the epidermis with a scalpel or a needle for examining the surface. For examining pressed specimen of stem, take separate tissue and press them with a scalpel on the slide.

IB. Powder

For examining characters of the powder take sufficient amount of powder in Chloral-hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time.

2. FRUIT AND SEEDS

2A. Entire materials

For microscopic examination of fruit and seed take the specimens or outer coat of seed or fruit and examine as described below :

i. Outer *Coat* — For examining the outer coat boil 3 or 4 seeds or fruits in caustic alkali solution in a test tube for 1-2 minutes (outer coat specimens with intensive pigmentation are boiled for longer period). After boiling, place the pieces on slide, remove the layers of the coat and examine them after mounting in glycerol solution.

ii. Section — If fruits or seeds are too hard to cut then boil them for 15-30 minutes or more depending on their hardness or keep them in moistening chamber or absorb in water and chloroform solution or soften them with stem and then cut the specimen for examining purpose.

For cutting small, flat seeds (which are difficult to hold) place them in a pith or potato slit for section cutting. Small, round or smooth seeds cannot be into section in the pith, then in such cases, they

may be embedded in paraffin wax blocks for section cutting. For this, a block of paraffin (0.6 x 0.5 x 1.5 cm in size) is made and the seed in embedded in the block by making a cavity or a pit in the block with a hot teasting needle. Cut the section with a sharp razor (through the object) together with the paraffin, place them on to the slide, remove paraffin with a needle or wash it with xylene and examine the section in *chloral-hydrate solution.*

2B. Powder

For examining the structure of the cells of the seed coat and the cells of the embryo take a small amount of powder of the material on a slide in glycerol and cover it with a cover slip and examine.

1. ***Starch*** — For examining the presence of starch in the seed, take two specimens, one in iodine solution and the other in water. With iodine solution starch turns blue. Shape and the structure of starch grains can be seen in water and their size is measured.

When examining objects containing starch, prepare specimen by slightly warming in chloral-hydrate solution.

2. ***Fixed oil*** — For examining the presence of fixed oil, prepare a specimen in a solution of Sudan III droplets of fixed oil are coloured orange pink. When examining objects containing small amount of fixed oil, prepare a specimen by slightly warming in chloral-hydrate solution, and when examining objects containing large amount of fixed oil, then the powder is de-fatted and clarified as follows:

Place 0.5 g. of the powder in a porcelain dish, add 5-10 ml. dilute nitric acid and boil for 1 minute, the strain *off* the liquid through a cloth, wash the residue with hot water and return it to the porcelain dish with a spatula, boil it with 5-10 ml of *caustic alkali solution* for 1 minute and again strain it through the cloth and wash with water. Examine the residue in a glycerol solution, after the treatment the structure of the layers of the coat and their cells can be seen very distinctly.

3. ***Mucilage*** - Prepare a specimen in Ruthenium Red and examine it under a light microscope or under dissecting microscope. Mucilage appears as pinkish-red or yellow coloured masses.

3. BARKS

3A. Entire material

Prepare transverse or longitudinal section of bark. To soften break it into pieces of about 1-2 cm long and 0.5-1 cm wide and boil with in a test tube for 1-3 minutes. Soft pieces are then straightened with a scalpel so as to have an exact transverse or longitudinal direction. Cut the section with razor; moisten the surface of the bark with glycerol solution. Remove the sections with a brush and place them on the slide. Thin pieces of the bark are cut by placing them in the pith (potato or carrot). The sections are treated with various reagents before examining.

1. ***Lignified elements*** — For testing lignin add several drops of *phloroglucinol* and a drop of *concentrated hydrochloric acid* to the section on a slide then draw off the liquid, and cover with a cover slip (the specimen should not be heated); the lignified elements are pink. The excessive stain can be washed out with acidified alcohol.

2. ***Starch*** — Starch is detected by treating with iodine solution.

3. ***Tannin*** - Tannin is detected by treating with *ferric ammonium sulphate solution* (blue-black or green black colour shows the presence of Tannin) or with *potassium-di-chromate solution* (brown colour indicates the presence of Tannin).

4. ***Anthraquinone derivatives*** - Anthraquinone derivatives are detected by treating with alkali solution (blood-red-color shows the presence of anthraquinone derivatives).

3B. Cut materials

Prepare small pieces or scraping of bark and boil them for 3-5 minutes in a solution *of caustic alkali* or *potassium hydroxide* or in *nitric acid solution* and then mount in *glycerin* for examination on a slide covered with a cover slip.

3C. Powder

Prepare specimen for examination by placing a little amount of powder on a slide, add 1-2 drops of *phloroglucinof* and a drop of concentrated *hydrochloric acid,* cover slip, draw off the liquid from one side of the slide with filter paper, and then apply 1-2 drops of *chloral-hydrate solution* from the other side of the slide, lignified elements are stained crimson-red. Specimen may also be prepared with *caustic alkali or ferric ammonium sulphate* for this purpose.

4. ROOTS AND RHIZOMES

4A. Entire materials

For anatomical examination of entire roots and rhizomes cut transverse and longitudinal sections. For this soften small pieces of roots without heating in *glycerol solution* for 1-3 days, depending on their hardness. The softened roots are straightened with the help of a scalpel in the right direction and then cut a section with the razor. First, cut thicker entire slices and then make thin, smaller sections. Stain the entire slices with *phloroglucinol* and *concentrated hydrochloric acid* or with *safranin* examine the specimen under a dissecting microscope. For micro-chemical test the small and thin sections are examined under microscope, as follows:

a. *Starch* - Starch is detected with iodine solution. For this, prepare specimen with water to measure the granule of starch with an ocular micrometer.

b. *Inulin* - Inulin is detected with Molish's reagent. For this place a little powder on a slide and apply 1-2 drops of naphthol and drop of concentrated sulphuric acid, if inulin is present, the powder will appear reddish-violet coloured. Starch also gives this test, so the test for inulin can be done in the absence of starch.

c. *Lignified elements* - Lignified elements (fibrovascular bundles, mechanical tissue etc.) are detected with *phloroglucinol* and concentrated *hydrochloric acid* or *safranine solution* as mentioned above for barks.

d. *Fixed oil* — For fixed oil detection use Sudan IV, as mentioned above for fruits and seeds.

If required for tannin, anthraquinone derivatives test as mentioned above.

4B. Cut material

Make small pieces or scrapping of roots or rhizomes and boil them for 3-5 minutes in *caustic alkali*, or in *nitric acid* and then make pressed specimen and immerse them in *glycerol*. Micro chemical tests can be performed with scrapings for various chemicals as mentioned above.

4C. Powder

Prepare several specimens of the powder on slides in *chloral hydrate solution* and perform the above mentioned standard tests for detection of starch, fixed oil, inulin, lignified elements, anthraquinone derivatives, tannins, mucilage, etc.

QUANTITATIVE MICROSCOPY

1. Determination of Stomatal Index

The stomatal index is the percentage ratio of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

Place transparent leaf fragments of about 5x5 mm in size on a microscope slide and prepare the mount, with a small drop of glycerol-ethanol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopic drawing apparatus is attached. Mark on the drawing paper a cross (x) for each epidermal cell and a circle (o) for each stoma. Calculate the result as follows :

Stomatal index=Sx100 / E+S

Where S = the number of stomata in a given area of leaf;

E = the number of epidermal cells (including trichomes) in the same area of leaf.

For each sample of leaf make not fewer than ten determinations and calculate the average index.

a. Type of Stomata

There are several types of stomata, distinguished by the form and arrangement of the surrounding cells. The following descriptions apply to mature stomata.

1. **Anomocytic** (irregular-celled) - Previously known as ranunculaceous. The stoma is surrounded by a varying number of cells in no way differing from those of the epidermis generally.

2. **Anisocytic** (unequal-celled) - Previously known as cruciferous or solanaceous. The stoma is usually surrounded by three subsidiary cells, of which one is markedly smaller than the others.

3. **Diacytic** (cross-celled) -Previously known as caryophyllaceous. The stoma is accompanied by two subsidiary cells whose common wall is at right angles to the guard cells.

4. **Paracytic** (parallel-celled) - Previously known as rubiaceous. The stoma has each side one or more subsidiary cells parallel to the long axis of the pore and guard cells.

5. **Actinocytic** - The stomata surrounded by epidermal cells uniformly arranged along the radii of a circle with the stomata at the sample.

2. Determination of palisade Ratio

Palisade ratio is the average number of palisade cells beneath one epidermal cell.

Transfer a fragment of transparent leaf to a microscopic slide and prepare the mount of the upper epidermis in chloral hydrate solution and put a small drop of glycerol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and 6x eye piece, to which a microscopic drawing apparatus is attached. Trace four adjacent epidermal cells on paper; focus gently downward to bring the palisade into view and trace sufficient palisade cells to cover the area of the outline of the area of the four epidermal cells. Count the palisade cells under the four-epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4; this is the "palisade ratio".

For each sample of leaf make not fewer than ten determinations and calculate the average number.

3. Determination of Vein-Islet Number

The mesophyll of a leaf is divided into small portions of photo synthetic tissue by anastomosis of the veins and veinlets; such small portions or areas are termed "Vein-Islets". The number of vein-islets per square millimeter is termed the "Vein-Islet number". This value has been shown to be constant for any given species and, for full-grown leaves, to be unaffected by the age of

the plant or the size of the leaves. The vein-islet number has proved useful for the critical distinction of certain nearly related species. The determination is carried out as follows:

For Whole or Cut leaves - The transparent leaf pieces are stained with *safranin solution* and prepare the mount in *glycerol.* Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the center of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make not fewer than three determinations and calculate the average number of vein-islets per square millimeter.

For Leaf Fragments having an area less than 4 square millimeters — Take fragments of leaf lamina each with an area of not less than 1 square millimeter, excluding the midrib and the margin of the leaf. Clear and prepare a mount as stated above. Use a IOx objectives and a 6x eyepiece and draw a line representing 1 mm on a sheet of paper by means of a microscopial drawing apparatus and construct a square on this line representing an area of 1 square millimeter. Carry out the rest of the procedure as stated above. The result obtained is the number of vein-islets in 1 square millimeter. For each sample of leaf make no less than 12 determinations and calculated the average number.

Determination of Stomatal Number

Place leaf fragments of about 5x5 mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transport. Transfer a fragments to a microscopic slide and prepare the mount the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side

of the cover glass to prevent the preparation from drying. Examine with 40x objective and a 6x eye piece, to which a microscopic drawing apparatus is attached. Mark on the drawing paper a cross (x) for each stomata and calculate the average number of stomata per square millimeter for each surface of the leaf.

Other Tests

Powder

The coarseness of fineness of a powder is classed according to the nominal aperture size expressed in μm. of the mesh of the sieve through which the powder will pass, and is indicated as follows.

Descriptive term	Particle size
Coarse (2000/355)	All the particles will pass through a No. 2000sieve, and not more than 40% through a No. 355 sieve.
Moderately coarse (710/250)	All the particles will pass through a No. 710 sieve, and not more than 40% though a No. 250 sieve.
Moderately fine (355/180)	All the particles will pass through a No. 355 sieve, and not more than 40% through a No. 180 sieve.
Fine (180)	All the particles will pass through a No. 180 sieve.
Very fine (125)	All the particles will pass through a No. 125. sieve.

References:

1. C.k.Kokate, A.P. Purohit, S.B.Gokhale., Pharmacognosy , Nirali prakashan, 19th ed.,2002.p.99
2. The Ayurvedic Pharmacopoeia of India, vol-1, part-II, Dept. of ISM, Ministry of Health and Family Welfare, Govt. of India, 1999, appedix-1, pp.
3. Anonymous., Manual for Quality control of Herbal Drugs, National Botanical Research Institute, Lucknow-1, 2001. Chapters 2, 3.

Conceptual study
Part III

5. Conceptual study Part- III

(Analytical Pharmacognosy)

Analytical pharmacognosy deals with establishing standard parameters of a crude drug with respect to Physical evaluation and Chemical evaluation.

PHYSICAL EVALUATION

Physical standards are to be determined for drugs, whenever possible. These are rarely constant for crude drugs, but may help in evaluation, specifically with reference to moisture content, specific gravity, density, optical rotation refractive index, melting point, viscosity and solubility in different solvents.

PREPARATION OF TEST SAMPLE

Withdraw as much as may be necessary of the original sample by quartering, taking care to see that the portion is representative of the gross sample. In the case of unground or un powdered drugs, grind the sample so that it will pass through a No. 22 sieve. If the sample cannot be ground, it should be reduced to as fine a state as possible. Mix by rolling it in paper or cloth, spread it out in a thin layer, and withdraw the portion for analysis.

1. FOREIGN MATTER AND DETERMINATION OF FOREIGN MATTER

A. FOREIGN MATTER

Drugs should be free from moulds, insects, animal fecal matter and other contaminations such as soil, stones and extraneous material.

Foreign matter is material consisting of any or all of the following: -

(1) In particular, parts of the organ or organs from which the drug is derived other than the parts named in the definition or for which a limit is prescribed in the individual monograph.

(2) Any organ or part of organ, other than those named in the definition and description. The amount of foreign matter shall not be more than the percentage prescribed in the monograph.

B. DETERMINATION OF FOREIGN MATTER

Weight 100 - 500 g of the drug sample to be examined, or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or the use of a lens (6x). Separate and weight it and calculate the percentage present.

2. DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING)

Proceeding set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or un powdered drug, prepared about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.

Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high-speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drugs in the tared evaporating dish dry at 105° for 5 hours, and weigh. Continue the drying and weighing at one-hour interval until difference between two successive weighing corresponds to more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.01 g difference.

3. DETERMINATION OF TOTAL ASH

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450° until free carbon, cool and weight. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°. Calculate the percentage of ash with reference to the air-dried drug.

4. DETERMINATION OF ACID INSOLUBLE ASH

Boil the ash obtained as above method for 5 minutes with 25 ml *of dilute hydrochloric acid;* Collect the insoluble matter in a Gooch crucible, or on an ash less filter paper, wash with hot water and ignite to constant weight, calculate the percentage of acid-insoluble ash with reference to the air dried drug.

5. DETERMINATION OF SULPHATED ASH

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool is a desiccators and weigh. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignite gently at first, unit the substance is thoroughly charred cool, moisten the residue with 1 ml of H_2SO_4, heat gently until white fumes are no longer evolved and ignite at 800°± 25 until all black particles have disappeared conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of H_2SO_4 and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighing do not differ by more than 0.5 mg.

6. DETERMINATION OF WATER SOLUBLE ASH

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°. Subtract the weight of the insoluble matter from the weight of the ash;
The difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

7. DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105° to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

8. DETERMINATION OF WATER SOLUBLE EXTRACTIVE

Proceed as directed for the determination of Alcohol-soluble extractive, using *chloroform •water* instead of ethanol.

9. DETERMINATION OF ETHER SOLUBLE EXTRACTIVE (FIXED OIL CONTENT)

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with *Solvent ether* (or petroleum *ether*, b.p. 40° to 60°) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105° to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

10. FLUORESCENCE TEST (CHASE & PRATT, 1949 WITH SOME MODIFICATION)

1. A small amount of powdered drug is placed on a micro slide and observed under UV 366, UV 254 and in daylight. The fluorescent characteristics of the powder, if any, is apparent. (as control).
2. A small amount of the powdered drug is suspended in a solution of nitrocellulose in amyl acetate on a micro slide. The solvent is evaporated and the slide observed after few minutes under UV 366, UV 254 and in daylight.
3. A small amount of the powdered drug is placed on a micro slide and treated with IN HC1 and observed under UV 366, UV 254 and in daylight while wet.
4. A small amount of the powdered drug is placed on a micro slide and treated with IN HC1 followed by nitrocellulose in amyl acetate and the slide observed after few minutes in daylight and under UV 254, UV 366.
5. A small amount of the powdered drug is placed on a micro slide and treated with IN NaOH and the slide observed after few minutes in daylight and under UV 254, UV 366.
6. A small amount of the powdered drug is placed on a slide and treated with IN NaOH and allowed to dry for approximately thirty minutes. Then a mounting fluid consisting of nitrocellulose in amyl acetate is added and the slide observed after a few minutes in daylight and under UV 366, UV 254.
7. A small amount of the powdered drug is placed on a micro slide and treated with IN NaOH in methanol and observed under UV 366, UV 254 and in daylight while still wet.
8. A small amount of the powdered drug is placed on a micro slide and treated with 50% KOH and observed under UV 366, UV 254 and in daylight.
9. A small amount of the powdered drug is placed on a micro slide and treated with 50% $H_2SO_4$and observed under UV 366, UV 254 and in daylight while still wet.
10. A small amount of the powdered drug is placed on a micro slide and treated with Cone. $H_2SO_4$and observed under UV 366, UV 254 and in daylight while still wet.
11. A small amount of the powdered drug is placed on a micro slide and treated with 50% HNO_3 and observed under UV 366, UV 254 and in daylight while still wet.
12. A small amount of the powdered drug is placed on a micro slide and treated with Cone. HNO_3 and observed under UV 366, UV 254 and in daylight while still wet.
13. A small amount of the powdered drug is placed on a micro slide and treated with acetic acid and observed under UV 366, UV 254 and in daylight while still wet.

14. A small amount of the powdered drug is placed on a micro slide and treated with iodine water and observed under UV 366, UV 254 and in daylight while still wet.

Note: - Observations are made most easily in a dark room but satisfactory results can be obtained merely working in a shaded comer of the laboratory if the slides are placed on a black background and are shielded from direct visible light.

11. EXTRACTION OF PLANT MATERIAL

Extraction involves the separation of bioactive portion of the plant tissues from the inactive components by using selective solvents in standard extraction procedures. The products so obtained are relatively impure liquids, semisolids or powders. These include classes of preparations known as decoctions, fluid extracts, tinctures, semi solid extract and powder.

Extraction of the powdered plant material is carried out in three ways. (i) Hot aqueous extraction or decoction. (ii) Cold percolation (iii) Hot continuous extraction or Soxhlet extraction

(i) Hot aqueous extraction (Decoction)

In this process the crude drug is boiled in specified volume of water for definite time, cooled and filtered. This method is suitable for extracting water soluble, heat stable constituents. Ratio of the drug to water is fixed e.g. 1: 4 and 1: 16 this is very commonly used in preparation of Ayurvedic extracts called Quath.

(ii) Cold percolation

This is the most frequently used method for extracting the active ingredients in the preparation of tinctures and fluid extracts. The powdered plant material is filled in a percolator and the specified solvent added to it in a quantity enough to wet the material and to give a shallow layer above the mass. The top of the percolator is closed and the mixture allowed to macerate in the closed percolator for 24 h. The outlet of the percolator is then opened and the liquid contained therein drained out and collected. This way four to five washes of solvent are given to plant material till it gets completely extracted. All these washes from the percolator are clubbed together and subjected to concentration.

(iii) Hot continuous extraction (Soxhlet extraction)

In this method, the finally powdered plant material is placed in porous bag or thimble made of strong filter paper and placed in chamber E. the extracting solvent in flask A is heated. Its vapors get

condensed in the condenser D. the condensed extractant drops into the thimble containing the crude drug, extracting it by contact. When the level of liquid in the chamber E rises to the top of the siphon tube C, the liquid contents of E siphon into A. This Process is continuous and is done until a drop of solvent from the siphon tube when evaporated does not leave a residue. The advantage of the method is that large amount of drug can be extracted with a much smaller quantity of solvent as compared to other methods of extraction.

The selection of the method and solvent to be used for extraction depends mainly on the nature of the constituents present in the material to be extracted:

If the biological activity lies in non-polar constituents then a non-polar solvent may be used, eg. Hexane is generally used for extracting lupeol an active constituent of *Crateava nurvala.* However if the active constituents are polar compounds like saponins (eg is *Bacopa monnieri)* then aqueous methanol or water may be used.

If the constituents are thermolabile, then cold percolation is preferred. But for thermostable constituents Soxhlet extraction is may be used.

Temperatures higher than required should be avoided while hot extraction.

Concentration and Drying

The solvent extract obtained above is concentrated under reduced pressure below 40°C using a rotatory evaporator. The concentrated extract is finally lyophilized to give a completely dry product.

Fractionation

The dried extract is then subjected to solvent fractionation using solvents of different polarities viz. hexane, chloroform, ethyl acetate, n-butanol and water. The different fractions are subsequently chromatographed to give pure bioactive compounds.

Isolation and Identification of Markers

The marker compounds are isolated using different techniques like preparative TLC, Column chromatography or preparative HPLC. The isolated compounds are identified using co - TLC, chemical degradation methods and spectroscopic methods including IR, NMR and Mass spectroscopy.

PHYTOCHEMICAL STUDIES FOR STANDARDIZATION OF HERBAL DRUGS (SINGLE AND COMPOUND FORMULATIONS)

It comprises of different chemical tests and chemical assays. The isolation, purification and idintification of active constituents are chemical methods of evaluation. Quantitative chemical tests such as acid value, saponification value etc., are also covered under this technique. Some of these tests are useful in evaluation of resins (sulphated ash, acid value); balsams (acid, saponification and ester values) volatile oils (acetyl and ester values) ; and gums (methoxy determination and volatile acidity). Preliminary phytochemical screening is a part of chemical evaluation. The qualitative chemical tests are useful in detection and adulteration. Colophony which is admixed as an adulterant for resins, balsams and waxes is detedcted by copper acetate. Halphen's test for cotton seed oil, Van Urk's reagent for ergot, Vitali's test for tropane alkaloids, Murexide test for purine bases, etc. are examples of specific tests.

The purity of curde drugs is ascertained by quantative estimation of active chemical constituents present in them. The method may be useful in determining single active constituent or the group of related constituents present in the same drug.

The conventional trtrimetic estimations as applicable to the estimation of alkaloids from crude drugs, ester and aldehyde contents of volatile oils, gravimetric methods etc., are the techniquesof chemical assay. The alkaloid drugs which routinely analysed for their total alkaloidal content and main alkaloid are opium (morphine), belladona(atropine) and ergot(ergometrine), ipecacuanha (emetine), nux-vomica(strchnine), cinchona (quinine), rauwolfia (reserpine) . Chemical assays are carried out for cineole in eucalyptus oil, aldehydes in lemon oil, carvone in caraway oil, and dill oil. The chemical assays are also extended to anthraquinone and cardiac glycosides, content of balsamic acids like benzoic acid and cinnalmic acid in halsam of tolu, balsm of peru and prepared storax. The application of physical methods involving instrumentation is common complintary feature of chemical assays.

I. QUALITATIVE ANALYSIS

General screening of the alcoholic and aqueous extracts of the plant material is carried out for qualitative determination of the groups of organic compounds present in them.

1. Alkaloids

(a) *Dragendorff's test:* Dissolve a few mg of alcoholic or aqueous extract of the drug in 5 ml of distilled water, add 2 M hydrochloric acid until an acid reaction occurs, then add 1 ml of Dragendorffs reagent, an orange or orange-red ppt is produced immediately.

(b) Hager 's test: To 1 ml of alcoholic extract of the drug taken in a test tube, add a few drops of Hager's reagent. Formation of yellow ppt confirms the presence of alkaloids.

(c) Wagner's test: Acidify 1 ml of alcoholic extract of the drug with 1.5% v/v of hydrochloric acid and add a few drops of Wagner's reagent. A yellow or brown ppt is formed.

(d) Mayor's test: Add a few drops of Mayor's reagent to 1 ml of acidic aqueous extract of the drug. White or pale yellow ppt is formed.

2. Carbohydrates

(a) *Anthrone test:* To 2 ml ofanthrone test solution, add 0.5 ml of aqueous extract of the drug. A green or blue colour indicates the presence of carbohydrates.

(b) *Benedict's test:* To 0.5 ml of aqueous extract of the drug add 5 ml of Benedict's solution and boil for 5 min. Formation of a coloured ppt is due to the presence of carbohydrates.

(c) *Fehling's test:* To 2 ml ofaq extract of the drug add 1 ml of a mixture of equal parts of Fehling's solution 'A' and Fehling's solution 'B' and boil the contents of the test tube for few min. A red or brick red ppt is formed.

(d) *Molisch 's test:* In a test tube containing 2mlofaq extract of the drug add 2 drops of a freshly prepared 20% alcoholic solution of naphthol and mix, pour 2 ml cone sulphuric acid so as to from a layer below the mixture. Carbohydrates, if present, produce a red-violet ring which disappears on the addition of an excess of alkali solution.

3. Flavonoids

In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of diluted hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

4. Triterpenoids

Liebermann-Burchard's test: Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of cone sulphuric acid. A violet colour coloured ring is formed indicating the presence of triterpenoids.

5. Proteins

(a) *Biuret's test:* To 1 ml of hot aq. extract of the drug add 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.

(b) Millon 's test: Dissolve a small quantity of aq. extract of the drug in 1 ml of distilled water and add 5-6 drops of Millon's reagent. A white ppt is formed which turns red on heating.

6. Resins

Dissolve the extract in acetone and pour the solution into distilled water. Turbidity indicates the presence of resins.

7. Saponins

In a test tube containing about 5 ml of an aq extract of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 min. Honey comb like forth is formed.

8. Steroids

(a) *Liebermann-Burchard's test:* Add 2 ml of acetic anhydride solution to 1 ml of petroleum ether extract of the drug in chloroform followed by 1 ml of cone sulphuric acid. A greenish colour is developed which turns to blue.

(b) *Salkowski Reaction:* Add 1 ml of cone sulphuric acid to 2 ml of chloroform extract of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer.

9. Tannins

To 1 - 2 ml of extract of the drug add a few drops of 5% FeCl solution. A green colour indicates the presence of gallotannins while brown colour tannins.

10. Starch

Dissolve 0.015g of Iodine and 0.075g of Potassium iodide in 5 ml of distilled water and add 2 - 3 ml of an aq extract of drug. A blue colour is produced.

II. QUANTITATIVE ANALYSIS STARCH ESTIMATION (Mont Gomery, 1957)

Spectrophotometric method

Prepare 10% homogenate of the plant tissue in 80% Ethanol. Centrifuge at 2000 rpm for 15 minutes. To the residue thus obtained, add 4 ml of distilled water, heat on a water bath for 15 minutes and macerate with the help of glass rod. To each of the samples, add 3 ml of 52% perchloric acid and centrifuge at 2000 rpm for 15 minutes. The supernatant thus obtained is made up to known volume (generally up to 10 ml or depending on the expected concentration of starch). Take 0.1 ml aliquot; add 0.1 ml of 80% phenol and 5 ml cone. H_2S0_4. Cool and then read the absorbance at 490 nm.

SUGAR ESTIMATION (Mont Gomery, 1957) [Spectrophotometric method]

Prepare 10% homogenate of the plant tissue in 80% Ethanol. Centrifuge at 2000 rpm for 15 minutes. The supernatant obtained is made up to known volume (generally up to 10 ml or depending on the expected concentration of sugar). Take 0.1 ml aliquot; add 0.1 ml of 80% phenol and 5 ml cone. H_2S0_4. Cool and then read the absorbance at 490nm.

TANNIN ESTIMATION

Extract 2 g powdered plant material with 100 ml distilled water on water-bath, filter and make up of the volume to 100 ml in volumetric flask. Take 1 ml aliquot of it, at add 5 ml of folin phenol reagent, 10 ml saturated sodium carbonate and make up the volume to 100 ml in volumetric flask. After half hours set the instrument to zero through blank and take the corresponding absorbance of different samples at 760 nm.

FATTY OIL ESTIMATION

To estimate fatty oils, extract accurately weighed air-dried powdered plant material with petroleum ether (40-60°C) in Soxhlet apparatus. Dry the extract over anhydrous sodium sulphate and remove the solvent under vacuum at 40°C. Weigh the residue and calculate the percentage with reference to the weight of plant material used.

DETERMINATION OF FOAMING INDEX

Reduce about 1 g of the plant material to a coarse powder (sieve size no. 150), weigh accurately and transfer to a 500ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100ml volumetric flask and add sufficient water through the filter to dilute the volume to 100 ml.

Place the above decoction into 10 stoppered test-tube (height 16 cm. diameter 16 mm) in a series of successive portions of 1, 2, 3, up to 10 ml and adjust the volume of the liquid in each tube with water to 10 ml. Stopper the tubes and shake them in a lengthwise motion for 15 seconds, 2 frequencies per second. Allow it to stand for 15 minutes. Note 1 cm height of the foam and calculate the foaming index by following formula.

Foaming index = 1000/a

Where 'a' is the volume in ml, of decoction used for preparing dilution in tube where foaming is observed.

PROTEIN ESTIMATION (Lowry *et al* 1951)

Homogenize 100 mg plant material with 3 ml of 10% Trichloroacetic acid. Centrifuge the homogenate at 10,000 rpm. Discard the supernatant. Treat the pellets obtained after centrifugation with 3 ml IN NaOH, heat on water bath for 7 minutes and cool. Centrifuge the solution again for five to ten minutes at 5000 rpm. To 0.5 ml of supernatant thus obtained after centrifugation, add 5 ml reagent containing 100 parts of 2% solution of sodium carbonate and one part of 2% solution of sodium potassium tartrate. Allow it to stand for ten to fifteen minutes. Then add 5 ml Folin and Ciocalteu's Phenol reagent (diluted with distilled water in ratio of 1:1) and allow standing for half-hour for development of colour and then finally measuring the absorbance at 700 nm.

METHOD FOR ALKALOID ESTIMATION

Macerate the plant material with 2% acetic acid in water, filter and concentrate the filtrate under reduced pressure at 45°C to one third of the original volume. Adjust the pH to 2 by 4 M HC1. The yellow precipitate will be separated from the solution

(A). Dissolve in it 0.1 M HC1 to give solution

(B). Add Mayor's reagent to the solution A and B to give precipitate of Alkaloid Mayer complex. Dissolve it again in acetone - methanol - water (6 :2 : 10) to give solution of Alkaloid Mayer Complex. Pass this complex finally through Amberlite IRA 400 anion exchange resin (500 g) to give an aqueous solution of alkaloid chlorides.

DETERMINATION OF MUCILAGE CONTENT

Extract 5 g of accurately weighed powdered drug with solvent ether in a Soxhlet apparatus for 6-8 hours. Dry the mass and macerate overnight with 50 ml of absolute alcohol and discard the supernatant liquid after maceration as well as the ether extract. Macerate again the defatted and decolorized powder for about 18 to 20 hrs with 50 ml of 5% aqueous acetic acid solution. Repeat the maceration process at least twice for complete extraction. Combine the acid extracts, filter and concentrate to about 50 ml by evaporation on a water bath. Precipitate the mucilage completely by adding excess of absolute alcohol. Filter the entire mixture through filter paper and wash the residue repeatedly with small volumes of absolute alcohol. Finally dry the filter paper and residue to constant weight at 100°C. cool in a desiccators and weigh.

DETERMINATION OF VOLATILE OIL IN DRUGS

The determination of volatile oil in a drug is made by distilling the drug with a mixture of *water* and *glycerin,* collecting the distillate in a in which the aqueous portion of the distillate is automatically

separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile is expressed as a percentage v/w.

The apparatus **(Clevenger's apparatus)** consists of the following parts. The apparatus described below is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.

(a) Distilling Flask - A spherical flask, 1,000 ml capacity with ground neck, taper of ground socket 1 in 10, internal diameter of larger end 34.35 to 34.65 mm.

(b) Still head - Graduated measuring tube, and return flow tube made in one piece, in accordance with the following specifications. External diameter of the smaller end 31.0 to 31.2 mm. Minimum length of the ground zone - 34 mm.

Tube AC, length - 220 to 240 mm.
Internal diameter - 13 to 15 mm.

Bulb CD, length - 100 to 110 mm.
Internal diameter - 13 to 15 mm.
Spiral condenser — ground joint accurately fitting in the ground neck of the tube EG, taper lin IO.
Tube **EG,** length - 80 to 90 mm.
Internal Diameter - 30 to 40 mm.

Bulb B - length 20 to 22 mm.
Internal diameter - 15 to 20 mm.
The distance between B and P is 120 to 125 mm. Junction P and the center of the bulb B must be in the same horizontal plane.

Measuring tube JL - length of the graduated portion 144 to 155 mm capacity 2 milliliters graduated into fifths and fiftieths of a milliliter.

Tube **PL** - return flow tube - Internal diameter - 7 to 8 mm.
Levelling tube I, length — 450 to 500 mm. Internal 10 to 12 mm. Tapering at the lower end with a wide top (20 to 25 mm diameter).

(c) **Burner** - A luminors Argand burner with chimney and sensitive regulative tap.
(d) **Stand** - A report stand with asbestos covered ring and clamp carrying a piece of metal tubing connected by a short length of rubber tubing with the water inlet of the condenser jacket.

The Whole of the apparatus is effectively screened from draught.

The apparatus is cleaned before each distillation by washing successively with *acetone* and *water*, then inverting it, filling it with *chlomic sulphuric acid* mixture, after closing the open end at G, and allowing to stand, and finally rinsing with water.

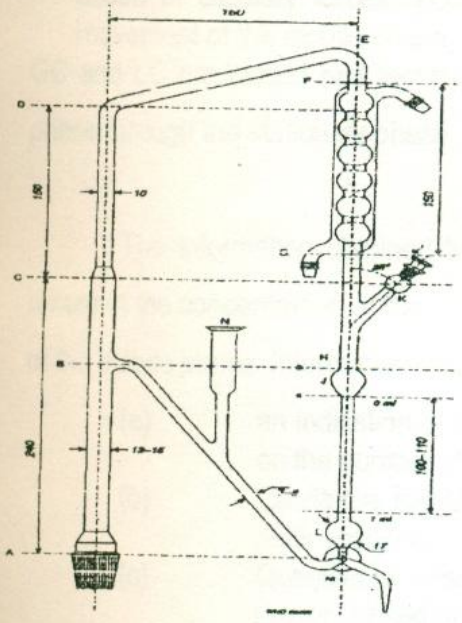
Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml *of glycerin* and 175 ml of - *water* in the one litter distilling flask, and a pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into graduated reciver, keeping the tap open until the water overflows, any air bubbles in the rubber tubing are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flack are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate which keeps the lower end of the condenser cooL The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap is opened and the tube lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the tap is closed and the volume is read.

The tube is then raised till the level of water in it is above the level of when the tap is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug. The dimensions of the apparatus may be suitably modified in case of necessity.



' For reasons of clarity, the 0.01-ml graduations are not shown on the tube jL

Apparatus used to determine volatile oils (dimensions in mm)'

IV. CHROMATOGRAPHIC TECHNIQUES

Chromatography is a physical method of separation in which the components to be separated are distributed between the two phases; one of these is a stationary phase bed and the other is a mobile phase which percolates through this bed.

It occurs as a result of repeated adsorption/ desorption during the movement of the sample components along the stationary bed, and the separation is due to differences in distribution constants of the individual sample components. The stationary phase includes solid, liquid coated on a solid support. The mobile phase includes liquid and gas.

Based on the stationary and mobile phase, the chromatographic technique can be of the following types:

1. Gas chromatography: the mobile phase is an inert gas and the stationary phase is either an adsorbent or a liquid distributed over the surface of a porous, inert support.
2. Liquid chromatography: the mobile phase is a liquid of low viscosity that is caused to flow through a bed of sorbent. The sorbent may be immiscible liquid coated on to a porous support, or an inert sorbent of controlled pore size.
3. Thin Layer Chromatography (TLC): a liquid mobile phase moves through a layer of sorbent by the action of capillary forces. TLC is an open bed technique as pressure is not required for the movement of the mobile phase.

GC and LC are closed bed techniques as pressure gradient is used in the movement of the mobile phase through the stationary phase.

The information obtained by the chromatographic experiment is called the chromatogram, a record of the concentration or the mass profile of the sample components as a function of the movement of the mobile phase. Information that can be extracted from a chromatogram include

(a) an indication of the sample complexity or the number of components present based on the number of peaks,
(b) Qualitative identification of the samples based on the accurate measurement of the peak positions,
(c) Quantitative assessment of the relative concentration or the amount of substance present based on the peak size.

The common chromatographic techniques used are Column chromatography. Paper chromatography, TLC, GC and HPLC

Column Chromatography

The principle underlying the separation of the compounds is their adsorption at the solid-liquid interface. For successful separation the compounds of the mixture should show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and the component must be reversible. As the adsorbent is washed with the fresh solvent, the various components move down the column and arrange themselves in the order of affinity to the adsorbent. Those with the least affinity move down the column at a faster rate than those with greater affinity.

Paper chromatography

Paper partition chromatography was developed as a technique for the separation of sugars and amino acids. Paper is used as a support or adsorbent, but partition plays a major role than adsorption in the separation of the components of mixtures as the cellulose fibbers have a film of moisture round them even in the air dry state. The technique is therefore allied to column partition chromatography.

TLC

The technique of TLC and paper chromatography are similar in that they both are open bed techniques in which substances are separated by the differential migration that occurs when a solvent flows along a thin layer of paper (PC) or a fine powder spread on glass or a plastic plate (TLC). For most substances, TLC offers a faster and a more efficient separation than PC. TLC has achieved phenomenal success not only in its use in analytical problems but also in preparative work for which thicker layers of coating is used.

HPTLC

In these the plates are coated with high performance silica gel, which are of very small and uniform in size (about 5pm). These high performance silica gels give more efficient and reproducible separation than conventional grades of silica gel. If small volumes of the samples are applied, then the spots are compact and the spots can be quantified with the help of the scanner using photo densitometry. The term HPTLC is used for the technique in which substances are accurately and precisely assayed using high performance grades of silica gel.

HPLC

The technique is based on the same modes of separation as classical column chromatography, i.e., adsorption, partition, ion exchange and gel permeation, but it differs from column chromatography in

that the mobile phase is pumped through the packed column under high pressure. The principle advantages of HPLC compared to classical column chromatography are improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity with which the separated substances may be quantified.

1. Thin-Layer Chromatography (TLC)

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference ! sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Apparatus

1. Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively, commercially prepared plated may be used.
2. An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.
3. The adsorbent or coating substance consisting of finally divided adsorbent materials, normally 5 μm to 40 μm in diameter, is suitable for chromatography. It can be applied directly to the plate or can be in bonded to the plate by means of Plaster of Paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualizing spots that absorb ultra-violet light.
4. A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

5. A storage rack to support the plates during drying and transportation.
6. A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that support the plates, back to back, with lid of the chamber in place.

7. Graduated micropipettes capable of delivering micro liter quantities say 10 μl and less.

8. A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

9. An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of plates - Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substances in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plated to dry in air, heat at 100° to 105° for a least 1-hour (except in the case plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow cooling, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs.

Method

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to from a layer of solvent 5 to 10 mm deep, close the tank and allow standing for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate.

Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the from of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Make the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow standing at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualize as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specified 'protected from light' or 'in subdued light' it is intended that the entire procedure be carried out these conditions.

Visualization

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (365 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term *secondary spot* means any spot other than the principal spot. Similarly, a *secondary band* is any band other than the principal band.

Rate of flow [Rf] Value

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance traveled by the spots by the distance traveled by the front of the mobile phase.

2. High Performance Thin Layer Chromatography (HPTLC)

TLC is used for the separation of simple mixtures where speeds, low cost, simplicity are required. HPTLC is an advanced versatile chromatographic technique for quantitative analyses with high sample throughput and is complementary to HPLC/GLC. It provides a chromatographic drug fingerprint. It is therefore suitable for monitoring the identity and purity of drugs. In HPTLC the various steps involved are

1. Application of sample
2. Chromatographic development
3. Detection of spots
4. Quantitation
5. Documentation

1. Application of sample

An automatic applicator (Linomat) is used for sample application. A known quantity of sample is dissolved in a known volume of solvent and the sample applied on percolated TLC plate either in the form of a spot or a band. However a band form is preferred because:

• Larger quantities of sample can be handled for application.

• Better separation because of rectangular area in which compounds are present on the plate

*Response of densitometry in better due to variable concentration of substances in a spot.

2. Chromatographic development (separation)

Development of the chromatogram is affected after the solvent of the applied sample is completely evaporated. Rectangular glass chambers or twin trough chambers are commonly used for TLC development.

3. Detection of spots

For densitometric scanning, detection under UV light is generally preferred. But post chromatographic derivatisation reactions are essentially required for detection when individual compounds does not respond to UV light or do not have intense fluorescence.

4. Quantitation and Documentation

Densitometry is *in situ* instrumental measurement of visible, UV absorbance, fluorescence quench directly. The scanner converts the spot/band on the layer into a chromatogram consisting of peaks similar in appearance to HPLC.

The portion of the scanned peaks on the recorder chart is related to Rf values of the spots on the layer and the peak height or area is related to the concentration of the substance on the spot.

3. High Performance Liquid Chromatography (HPLC)

HPLC is one of the latest analytical techniques, which is very essential for both quantification and standardization of the herbal materials. The technique is based on the same modes of separation of column chromatography, i.e., adsorption, partition, ion exchange and gel permeation, but it differs from column chromatography in that the mobile phase in HPLC is pumped through the packed column under high pressure. The principle advantage of HPLC when compared to classical column chromatography is improved resolution of the separated substances, faster separation times and the increased accuracy, precision and sensitivity with which the separated substances may be quantified.

The HPLC techniques involves the following:

Mobile phase: The HPLC technique is generally an isocratic elution technique, in which the same solvent system, which may be single or a combination of solvent is used through out the separation procedure. The selection of the mobile phase is one of the crucial steps in HPLC:

Pump: There may be one or more pumps, which are essentially used in pumping the mobile phase through the packed column under pressure.

Injection: The sample is injected (10-20μl) into the injection port in the load position and then after injection the lever is pulled back to inject position.

Column: The column used can be of two types normal phase and reversed phase. Generally reverse phase column is used. The column is chosen based on the composition of the mobile phase. If polar mobile phase is used then reverse phase column is used of which C18 is generally used. If the mobile phase is non polar then normal phase is used.

Detector: The detector is device, which detects the presence of substance and gives the absorbance of the compounds present. The detector may be a dual wavelength detector, which measures the absorbance at 2 wavelengths.

Recorder: Recorder is a computer, which records the absorbance of the substances and gives out the results in the form of peaks.

Interpretation: The peaks are then integrated, mainly valley-to-valley and the report contains the retention time, height of the peak and the % Area under the peak. With the Retention Time (RT) we can identify the substance, as the RT is characteristic of the substance under the given conditions. With the area and the height, the amount of the substances can be calculated.

Mobile phase → pump→ injection→column→ detection→ recorder

References:

1. C.k.Kokate, A.P. Purohit, S.B.Gokhale., Pharmacognosy , Nirali prakashan, 19th ed.,2002
2. The AyurvedicPharmacopoeia of India, vol-1, part-II, Dept. of ISM, Ministry of Health and Family Welfare, Govt. of India. 1999. appedix-1. appendix- 2
3. Anonymous., Manual for Quality control of Herbal Drugs, National Botanical Research Institute, Lucknow-1, 2001. Chapters 2, 3.
4. Trease & Evans Pharmacognosy, Wb Saunders Company Ltd.

Conceptual Study
Part IV

6. Conceptual study -Part- IV
(DETERMINATION OF CONTAMINATION)

Contamination of crude drugs can occur at different levels. The indiscriminate use of pestisides is resulting in presence of these harmful chemical in a dangerous proportions. The same is the case with microbial contamination which can result in spread of diseases . all these generally occur due to falty methods of, collection, cultivation, harvesting, processing defects, and faulty storage practices (bad manufacturing conditions etc.)

Considering all these aspects it becomes inevitable to test the given sample for levels of heavy metals, pesticides and Microbe so that they can be checked effectively in case they are present in large quantities than the prescribe d once by the organizations like WHO and the govt. agencies. The following are details of various tests to detects the contamination:

DETERMINATION OF HEAVY METALS/ PESTICIDE RESIDUES

HEAVY METALS – Lead, Copper, Cadmium, Chromium, Cobalt, Manganese, Iron, Nickel etc. metals are estimated by the following method.

Wash the plant in fresh running water to eliminate dust, dirt and possible parasites and then wash again with deionized water. Dry the sample in oven at 80°C. Digest 1g of each completely dried powdered sample in concentrated nitric acid and perchloric acid (3:1) until a clear solution is obtained. After adequate cooling, reconstitute the solution up to the desired volume i.e. 25 ml with deionized water and store in test tubes. Take all necessary precaution to avoid possible contamination of the sample. Analyze prepared samples on atomic absorption spectrophotometer. Hollow cathode lamps are employed for detection of heavy metals. Use standard reference material to provide calibration and quality assurance of each analytical batch. The efficiency of digestion of plant samples and test concentrations is determined by adding standard reference material of metals in plant samples.

PESTICIDE RESIDUE

Excessive usage of pesticides on the crops, which are meant for human consumption, is causing severe health hazards. World Health Organization (WHO) has provided the skeletal protocol to estimation pesticide residues in the plant materials which is as follows.

Grind the plant material into fine powder. Take 20-25 g. of the powder add acetonitrile water mixture (650: 350) and blend for 5 min. at high speed and filter. Transfer the filtrate into one liter separating funnel and add 100 ml. of light petroleum. Shake the apparatus for one to two minutes and add 10 ml. of sodium chloride (400g/L) and 600 ml. of distilled water. Shake the separating funnel vigorously for 30-45 sec. and allow the solvent layer to get separated. Collect the petroleum ether layer, wash it with water (thrice) then treat with anhydrous sodium sulfate. The extract is subjected to column chromatography. The column is packed with florosil, which is previously activated at 650° C., and the column is eluated with petroleum ether. Collect three fractions of 200 ml. each.

The first fraction contains chlorinated pesticides like aldrin, benzene hexa chloride, DDT, hepatachlor, lindane, methoxy chlor etc. while the second eluate contains dieldrin, endrin and phosphated pesticides. The third eluate contains phosphate pesticides (malathion).

The eluted samples are subjected to either gas chromatography or the eluate is combusted and subjected to spectrophotometry.

General aspects on the analytical methodology.

(i) The treated samples should be processed as earliest as possible.
(ii) Samples should be stored in air tight containers under refrigeration.
(iii) Solvent should be remove from the sample before storing. The process of concentrating solution/solvents should be made with great care, especially during the evaporation of the last traces of solvent in order to avoid loses of pesticide residues.
(iv) Samples should be protected from the light.
(v) All the solvents and reagents used in the analytical methods should be free of all foreign matter.
(vi) Maximum limit of pesticide residues for medicinal plant materials:

Limits can be determined by using the following formula

$$\frac{\text{API} \times 60 \text{ kg. (bw)} \times \text{extraction factor}}{\text{MDIx Safety factor } 100}$$

ADI = Acceptable daily in take, value of FAO/WHO.

bw = Body weight MDI= Mean daily in take of drug

DETERMINATION OF MICROBIAL COUNT

Medicinal plant materials generally carry a great number of bacteria and moulds. Current practices of harvesting, handling and production often cause additional contamination and microbial growth. There are some microbes seen in the plant materials which are pathogenic to the human being , e.g. *E. coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus,* certain type of yeast and moulds. Protocols for the identification and quantitation for the *E. coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus,* etc. are given here. Presence of these microbes beyond certain limits causes severe health hazards. Determination of microbial count is explained as per WHO guide lines.

ISOLATION AND IDENTIFICATION OF MYCOFLORA ASSOCIATED WITH CRUDE DRUGS

In order to record the fungal flora associated with crude drugs, blotter test as well as agar plate method would be followed. Hundred replicates of each sample would be surface sterilized with 0.2% sodium hypochloite solution for ten minutes. Subsequently the samples would be thoroughly rinsed with sterile distilled water and place in petri dishes, containing three layered moistened blotter paper and potato dextrose agar medium. The plates would be kept in BOD incubator for 7 days at 25 ±1 °C. The samples would be regularly observed from 3rd of incubation. Once the growth of fungi would be established, the fungi would be isolated aseptically transferring the small quantities of inoculum (fungal spores/mycelium) to potato dextrose agar slants. These slants would be incubated at 25 ±1 °C for growth and sporulation. The cultures of different fungi would be purified by single spore / hyphal tip method. Percentage incidence of fungi would be determined on the basis of occurrence of particular species in hundred replicates per sample.

References:

1. C.k.Kokate, A.P. Purohit, S.B.Gokhale., Pharmacognosy , Nirali prakashan, 19th ed.,2002
2. The AyurvedicPharmacopoeia of India, vol-1, part-II, Dept. of ISM, Ministry of Health and Family Welfare, Govt. of India, 1999, appedix-1, appendix- 2
3. Anonymous., Manual for Quality control of Herbal Drugs, National Botanical Research Institute, Lucknow-1, 2001. Chapters 2, 3.
4. Trease & Evans Pharmacognosy, Wb Saunders Company Ltd.

Materials and methods

part I

7. Materials and Methods Part-I.

Pharmacolinguistic and Botanical Standardization

History of Shallaki and Kunduru (Historical review)

The history of this drug is involved in much obscurity, owing to its having been confounded by both native and European writers with true Frankincense and Bdellium.

Sanskrit writers may possibly sometimes allude to it when they speak of Kunduru, but as this word is evidently the same as the Arabic Kundur, it is much more likely that they allude to the true Frankincense imported from Africa and Arabia, and which we know to have been introduced into India at a very remote period.

Mohammedan writers have probably included the produce of B.serrata among the different kinds of Mukul for, which they gave as the Indian synonym Guggul. It seems probable that the true Sanskrit name for B. serrata is Shallaki, from which the Hindi word Salai has been derived. The exudation from the tree is called Sallaki-drava or Sihla, and Guggulu.

Ainslie notices B.glabra as producing Guggulu, and B. serrata the olibanum of commerce, but the latter Salai, and quotes Dr. F.Hamilton's MS account of Shahabad, where the tree is said to be very common and to yield a resin called Sale-gond of Sale-lassa, which is not used.
Dr. Hamilton describbes it as of the consistence of turpentine when it flows from the tree; in this it is called at Chandalghar Gandhah-biroza, and in the dry state Sukha-biroza. (Mat. Ind.1.226)

Other European authors make the same mistake with regard to the source of commercial olibanum. B.glabra now considered to be only a variety of B. serrata. B. serrata is one of the commonest trees in some parts of Khandesh, Lonawara, and other neighbouring territories; the gum-resin is obtained by incising the bark. Dr. Hooker when ascending from Belcuppee in Bihar to

the height of 1.360 feet, came upon a small forest of these trees, which he lidens to the mountain ash.

Dr. Irvine remarks that the tree is very plentiful in the Ajmeer hills, where the gum resin is called Gandha-biroza, and is similar in appearance to Venice turpentine. Dr. O'Shaughnessy obtained fine specimens from the Shahabad country. The collection of Guggul is a source of revenue to the Bhils, and a stake cut from the tree is set in the ground when a marriage takes place is set in the ground when marriage takes place among them.

Sansktit writers describe Guggulu as moist, viscid, fragrant, and of a golden colour when freshly exuded- a description which is not applicable to the exudation of Balasmodendron , but is exactly so to the exudation of the B. serrata.

Olibanum trade[1]

The olibanum trade between Arabia and India probably dates from pre-historic times. In the book of Genesis (B.C. 1700)Arab merchants are mentioned as bringing spices, resin and atacte upon their camels from Gilead to Egypt; as no spices are produced in Arabia these must have come from India across the Persian Gulf. Alexander, B.C. 325 , found a vessel loaded with frankincense at the mouth of the Indus, and trading companies are mentioned in Yajnavalkya's code, 300 B.C.

The chief centers of trade on the western coast were Sopara, Sanjan, Chaul (Perimula), and Thana, where Markets appear to have been held during the fair season for the sale of Indian and Chinese merchandise in exchange for that of the West. Trade suffered from the opposition of Persians and Brahmins in the 6th century B.C., but recovered in the 2nd century B.C., when its course was still from Egypt to Berenike and Aden, found the Arabian coast to Kurrachi, and via Broach to the Thana ports. On the Roman conquest of Egypt, B.C., 30, trade greatly increased by te same route, until A.D., 47 Hippalus discovered the monsoons and the possibility of crossing direct to the Indian Coast. In Pliny's time the principle exports from India were seasamum, oil, sugar, spices, rice, nard, costus, pepper, lac and indigo and the imports frankincense, gum, storax, and Yavan girls (Shakuntala).

In A.D.525, cloves and aloe and sandalwood came from Ceylone, which had then become center of trade in the East . The trade between India and Egypt began to fall off about the close of the 3rd century, and by the 6th century it had almost entirely been transferred to east African ports.

Thana and its ports were still important marts from the 9th to the 13th century, as we learn from the accounts of El Beruni, Ibn Haukal and other Arabian travellers. (Bombay Gazetter).

Sanskrit writers speak of olibanum as Kunduru, and describe its use as an incense and as a local application to indolent swellings to promote suppuration.

The mohammeden writers describe several kinds of Olibanum:

1. Deep yellow tears, called Kundur Zakar, or Male frankincense
2. pale tears called kundur Unsa , female frank incense
3. Kundur madahraj, artificial tears made by shaking the moist exudation in a basket
4. Kishar Kundur or Kashfa the bark or scurf of the tree is cated with the exudation (Dhup of Bombay market)
5. Dukak Kundur, or dust of Olibanum. The first kind is most esteemed.

Mir Mohammad Husain says that Frankincense should burn readily, showing that it is not mixed with gum Arabic; should not emit much smoke, showing its freedom from Juniper resin. Moreover he remarks that a kind of Frankincense is said to be produced in India which has a reddish tinge probable an allusion to the gum resin of *Boswellia serrata*.Roxb.

Olibanum is considered by the Mohamedans to be hot and dry and to have desiccative, astringent and detergent properties. It is used internally and externally in much the same way as we use the products ot the Pines and Firs. In 1868, Olibanum was made official in the Pharmacopoeia of India, where it is recommended in chronic pulmonary affections, such as bronchorrhoea and chronic laryngitis, employed both internally and in the form of fumigation. In the same work an lintment has been introduced which is said to be a good stimulant application to carbuncles, ulcerations, boils, etc. A good imitation of commercial Burgundy Pitch may be made by incorporationg melted olibanum with water in a steam bath;

Bombay is the centre of the Olibanum trade. The houses which deal in gums have agents in Rabia and Africa, who buy ith up and forward ith in a mixed condition. It passes through the custom House as Esesh (a corruption of vishes), and is next sorted into four or five different qualities. The first, consisting of all the large clean tears, is destined for the European market. The intermediate qualities and the last, which is only the dust and refuse, supply the Indian and China requirements, the Kishar Kundur or Kashfsa of the Arabs forms a distinct article of commerce under the Indian name Dhup. The method of collection olibanum in Africa has been described by Cruttenden. (Trans, Bom. Geograph. Coc. VII., 1846, 121) Carter in the same publication has deacribed the collection of the drug in Southern Arabia. In both localities a simple incision in the tumid bark is made, and the product collected as soon as it becomes sufficiently hard. The cokkection is carried on from March to september in Africa, and from May to December in Arabia. Halfour found several species of Boswellia growing on Socotrs. Of these B. ameero yields an olibanum which on examination by Prof. Dobbie and Dr. Henderson was found to have the same composition as Arabian olibanum. The stalactitic from of olibanum, called Luban Moyeti, prodeced by B. frereana, Birdw is occasionally met with in the Bombay market under the name of Pandhri Esesh or Pandhri Luban; it differs from ordinary olibanum inasmuch as it contains gum soluble in water.

Shallaki is a very prominent drug in use from Samhita kala. Its uses are mentioned in all the three bhritrayi and the later texts followed them. It is not mentioned in any Vedic literature and seems to be not a drug in use at that period. References of its use as mentioned at various contexts from the Bhritrayi and contemporary Sanskrit literature are as follows:

Charaka Samhita [2]:

It is included in Purishavirajaneeya gana (C. Su. 4/32), Shirovirechaneeya gana (C.Vi. 8/151), and in Kashayaskanda (S.Vi.8/144). It is used in the kalpas such as balataila (C.Chi.28/153), *Amritadya taila* (C.Chi.28/161). The plant finds a place in the Jangala pradesha (C.Kal.1/7)trees and is aptly true for its habitat as studied now. An Amayika prayoga for swasha is recorded in Charakasamhita (C. Chi.18/114).

Susrutasamhita[3]:

This drug has been included in Rodhradi gana(S.Su.37/14-15), Eladi gana (S.Su. 37/24-25), Nygrodhadi gana(S.Su.37/48-49) and in Kashaya skanda (S.Su.43/18). It is recommended for Various amayika prayoga such as:

Vranaprakshalana(S/Chi.19/42),

Pittabhishyanda(S.U.10/7),

Atisara(S.U.40/96),

Raktatisara(S.U.40/119) and in

Swasha (S.U51/51).

Astanga Samgraha[4]:

It is included in Purisha virajaneeya gana (AS.Su.15/32), Eladi gana(AS.Su.16/37-38). It is used in Bala taila preparation useful for Vatavyadhi (AH.Chi.22/77).

Chakradutta[5]:

He has Mentioned in the preparationof Eladi taila (vatavyadhi Chikitsa-95).

Review from non-medical Sanskrit literature[6]:

i. Shallaki is mentioned in IV. 44 of Vikramorvasheeya of Kalidasa- editd by Velankar, new Delhi;

ii. Kumarasambhava-VIII. 33., - Elephants feed on the leaves of this plant (Gajabhakshya), emits sweet smell when broken;

iii. It is one of the important dravya-which should be collected and stored in a forte – Matshyapurana-217.65, 218.2;

iv. Mentioned in Vanoushadi varga of Amarakosha(112, 124);

v. Upavanavinoda.10

Pharmacolinguistic[7] and Botanical identification

(Critical review of the Literature)

A. Classification of the drug

The references from the Sanskrit medical texts are enlisted and categorized to recognize the important groups under which this drug is mentions which generally reflects the medical properties of the drug. The Sanskrit verse and the analogy are enlisted as follows:

Table No.1 list of Classification.

S.No.	Name of the Text referred	Gana/ Varga (Category)	References (Shlok No. etc)
1.	Charaka Samhita	Purishavirajaneeya Varga	Ch.Su. 4/32
		Kashaya Skanda	Ch.Vi. 8/141
		Shirovirechaneeya gana	Ch.Vi. 8/151
2.	Susruta samhita	Rodhradi gana	Su.Su.37/14-15
		Eladi gana	Su.Su.37/24-25

		Nygrodhadi gana	Su.Su.37/48-49
		Kashaya Skanda	Su.su. 43/18
3.	Astanga Sangraha	Vidvirajaneeya gana	AS.Su. 15/32
		Eladi gana	AS.su. 15/37-38
4.	Bhavaprakash Nighantu	Karpuradi varga	Karpuradi varga-41
5.	Raja Nighantu	Chandanadi	R.N .113-114
		Pushpadi varga	R.N. 135-137
		Amradi varga	R.N. 194-195
6.	Kayyadeva Nighantu		K.N. 861-863
7.	Kayyadeva Nighantu	Oshadi varga	K.N-1376-1377
8.	Shadrasa Nighantu	Kashaya skanda	P. 78.
9.	Madanapala Nighantu		MN-45-47.

<u>पुरीषविरजनीय गण</u>

जम्बु शल्लकीत्वक्कच्छूरामधूक शाल्मली श्रीवेष्टक मृष्टमृत्पयोस्योत्पलतिलकणा इति दशेमानि पुरीषविरजनीयानि भवंति ।। च.सू. 4.3.

<u>लोधादि गण</u>

लोध्र सावरलोध्र पलाश कुटन्नटाशोक फंजी कट्फलैलवालुक शल्लकीशल्लकीजिन्गिनी कदम्बसालाः कदली चेति ।। सु.सू.38.14.

एष रोध्रादिरित्युक्तो मेदः कफहरो गणाः ।
योनिदोषहरः स्तम्भी वर्ण्यो विषविनाशनः ।। सु.सू.38.15

<u>एलादि गण</u>

एलातगर कुष्ठमांसीध्यामक त्वक पत्र नागपुप्प प्रियंगु हरेणुका व्याघ्रनखशुक्तिचण्डास्थौणेयक श्रीवेष्टक चोच चोरक वालुक गुग्गुलु सर्जरस तुरुष्क कुन्दुरुकागरु स्पृक्कोशीर भद्रदारु कुंकुमानि पुन्नाग केशरं चेति ।।
सु.सू.38.24.

एलादिको वातकफौ निहन्त्याद्विषमेव च ।
वर्णप्रसादनः कण्डूपिडकाकोठ नाषनः ।। सु.सू.38.25

<u>न्यग्रोधादि गण</u>

न्यग्रोधोदुम्बराश्वत्थ प्लक्ष मधुककपीतन ककुभाम्र कोशाम्र चोरक पत्रजम्बूद्वय प्रियाल मधूकरोहिणी वंजुल कदम्ब बदरी तिन्दुकी सल्लकी रोध्र सावररोध्र भल्लातक नन्दी वृक्षश्चेति ।। सु.सू.38.48

न्यग्रोधादिर्गणो वण्य संग्राही भग्नसाधकः ।
रक्तपित्तहरो दाहमेदघ्नो योनिदोषहृत् ।। सु.सू.38.49.

विड्विरजनीय गण

जम्बूशल्लकि मधुकं नीलोत्पल कच्छुरा तिलच्छयाहम् भृष्ट्वा च मृत्पयस्या स शाल्मलीविड्विरजनानि ।। अ.सं.सू.15.32

एलादि गण

चण्डा गुग्गुलु देवधुपखपुराः पुन्नाग नागाद्वयोः ....
एलादिको वातकफौ विषं च विनियच्छति ।
वर्णप्रसादनः कण्डू पिटिका कोठ नाशनः ।। अ.सं.सू. 15.37-38.

Synonyms:

The method of nomenclature adopted in olden days were " multinomial" with each plant part being described with a unique set of names. Each name characterize a particular feature of the plant. When interpreted and composed together the names give the complete profile of the plant. All those synonyms of Shallaki are collected and presented below :

Interpretation of synonyms:

Basonym: a) Shallaki – 'सल गतौ लक् आस्वादने' – It tastes very good.

b) Kunduru – name stands for Shallaki niryasa

Appearance : *Hladini*-pleasing, *Vanya*- regular forest tree, *Maharuha*- big tree, *Mahataru*-big plant etc.

Leaves : *Deerghapatra*- Long leaves

Vanaparnika, Nimbapatrasyat- Leaves resembling Neem leaves.

Seeds : *Trysra Phala*,

Niryasa: *Surabhi*- - smells very good,

Suvaha – सुवहति it spreads in all directions,

Hladini, Hrada – It is very pleasing,

Susrava, -It oozes out in a nice manner,

Mocha, Pita, Piccha, Kunduru , Mutrika, Aswamutri etc.

Others:

Gajabhakshya – गजैः भक्ष्यते / it is eaten by Elephants

Rasa – रसं आस्वादने – it tastes good/ or it improves the taste by clearing the mouth.

Kundururka- कुन्दुरुरिव प्रतिकृति /

Table no. 2. Compilation of Synonyms of *Shallaki*

S.no.	Synonym	C.S	S.S	A.S/A.H	D.N	R.N	M.N	B.N	K.N	S.N
1.	Shallaki	+	+	+	+		+			
2.	Sallaki									+
3.	Surabhi									+
4.	Hradini/Hladini				+					+
5.	Yadomutra									+
6.	Gajapriya									+
7.	Maherana									+
8	Rasa									+
9.	Mocha/Mochi						+			+
10.	Gandhapatra									+
11.	Suradru									+
12.	Hrada/Hlada				+				+	
13.	Susrava				+	+			+	
14.	Suvaha								+	
15	Vanya								+	
16	Maheranya/Maheana Maharuha				+		+		+	
17.	Aswamutrika				+				+	
18.	Gandhakari								+	
19.	Vallaki				+				+	
20.	Vanaparnika						+		+	
21.	Gajabhakhya				+		+		+	
22.	Satikta								+	
23.	Atirasa								+	
24.	Gandhavira						+			

Table no. 3. Compilation of Synonyms of *Kunduru [Shallki niryasa]*

1.	Khapura								+	
2.	Kunduru	+	+	+	+	+	+	+	+	
3.	Kundu/Knda						+		+	
4.	Mukundaha								+	
5.	Bhishano				+		+		+	
6.	Bali				+		+		+	
7.	Nagaha								+	
8.	Shrivallahba								+	

9.	Swaksha								+	
10.	Medka								+	
11.	Kechuka								+	
12.	Kunduruki				+		+			
13.	Shikhari				+	+				
14.	Kundragopura				+					
15.	Sukundra				+					
16.	Teekshanagandha				+	+				
17.	Palindaha				+	+				
18.	Sourastra					+				
19.	Gopuraka					+				
20.	Bahugandha					+				
21.	Gandhavira						+			

Compilation of Sanskrit references from various Nighantu.

कर्पूरादि वर्ग [8] :

कुन्दुरुः मधुरस्तिक्तः तीक्ष्णस्त्वच्यः कदुर्हरेत् ।
ज्वरस्वेदग्रहालक्ष्मी मुखरोग कफानिलान् ।। भ.नि-कर्पूरादि.41.

चन्दनादि वग [9]:

वुन्दुरुक : सौराष्ट्रः शिखरी कुन्दुरुक कुन्दकस्तीक्ष्णः ।
गेपुरको बहुगन्धः पालिन्यो भीषणश्च दशसंज्ञः ।।रा.नि.113।।

कुन्दुरुर्मधुरस्तिक्तः कफपित्तार्तिदाहनुत् ।
पाने लेपे च शिशिरः प्रदरामयशान्तिकृत् ।।रा.नि.114।।

वुन्दुरुप्कुन्दुरो रुण्डी दीर्घपत्रः खरच्छदः ।
रसाभः क्षेत्रसंभूतः सुतृप्णो मृगवल्लभः ।। रा.नि.118।।

गौल्यं कुन्दुरुमूलंच शीतं पित्तातिसारनुत ।।
प्रशस्तं शोथनानॉच बलपुष्टि विवर्धनम् ।।रा.नि.119।।

पुष्पादि वर्ग[10]:

नलश्लेप्मादि सुरभिः सुखवा च सा ।
अश्वमूत्री कुन्दुरुकी गजभक्ष्या ।। रा.नि.135।।

सल्लकी स्यात्कषायाऽतिशीता वीर्यो प्रकीर्तिता ।
बलासं हन्ति वातस्य प्रकोप शमनी मता ।। रा.नि.136।।

अन्यश्च सल्लकी सुरभिस्तिक्ता कषाया ग्रहणी रसा ।
कुष्ठासकफवातार्शो व्रण दोषर्विनाशिनी ।। रा.नि.137 ।।

आम्रादि वर्गः [11]
सल्लकः सल्लकी सल्ली सुगंधा सुरभिस्रवा ।
सुरभिगजभक्षा च सुवहा गज वल्लभा ।। रा.नि.195 ।।

गन्धमूला मुखामोदा सुश्रीका जलविक्रमा ।
हृद्या कुण्टरिका चैव प्रोक्ता यस्फला च सा । ।। रा.नि.194 ।।

छिन्नरुहा गन्धफला ज्ञेया चाष्टादशाह्वया ।
सल्लकी तिक्तमधुरा कषाया ग्रहिणी परा ।
कुष्ठास्रकफवातार्शो व्रणदोषार्ति नाशिनी ।। रा.नि.195 ।।

धन्वन्तरि निघण्टु
[12]कुन्दुरुः स्यात कुन्दुरुकः शिखरी कुन्द्रगोपुरः ।
सुकुन्द्रस्तीक्ष्णगन्धश्च पालिन्दो भीषणो बली ।।ध.नि.118

कुन्दुरुः कटुकस्तिक्तो वातश्लेष्म मलापहः ।
पाने लेपे च शिशिरः प्रदरामय शान्तिकृत् ।। ध.नि.119

शल्लकी वल्लकी हादा सुरभिः सुस्रवा च सा ।
अश्वमूत्री कुन्दुरुकी गजभक्ष्या महेरणा ।। ध.नि.122

शल्लकी स्यात कषायाऽतिशीता वीर्यो प्रकीर्तिता ।
बलासं हन्ति पित्तस्य प्रकोपशमनी मता ।। ध.नि .123

मदनपाल निघण्टु

[13]कुन्दुरुर्मोचकः कुन्दः खपुरो भीषणो बली ।
कुन्दुरुः स्वेदपवन श्लेष्म ब्रध्नज्वरापह ।। म.नि.45

शल्लकी वल्लकी मोची गजभक्ष्या महारुहा ।
गन्धवीरा कुन्दुरुकी सुस्रवा वनपर्णिका ।
शल्लकी व्रणपित्तास्र श्लेष्म पित्तातिसारजित ।। म.नि.47

कय्यदेव निघण्टु
[14]शल्लकी सुरभिर्मोचा सुगन्धा ह्लालादिनी हृदा ।
सुस्रवा सुवहा वन्या महेरण्याश्वमूत्रिका ।। क.नि.861

गन्धकारी गन्धवीरा वल्लकी वनपर्णिका ।
गजभक्ष्या कुन्दुरुकी सतिक्तातिरसा रसा ।। क.नि.862

शल्लकी तु वरा शीता क्रमेण रस वीर्ययोः ।
बलास पित्त पक्वातिसार व्रण विनाशिनी ।। क.नि.863
तत्पुष्पं कफवातास्र कुष्ठारोचक नाशनम् ।

<u>ओषधि वर्गः</u> [15]
खपुरः कुन्दरुः कुन्दुः मकुन्दुः भीषणो बली ।
नागः श्रीवल्लभः स्वाक्षो मोदुको केचकः परः ।। क.नि.1376

वुन्दुरुर्मधुरस्तिक्तः तीक्ष्णस्त्वच्यः कदुहरेत् ।। क.नि.1377
ज्वरस्वेदग्रहालक्ष्मी मुखरोगकफानिलान् ।

शल्लकी तुवरा शीता श्लेष्मपित्तातिसारजित् ।
रक्तपित्तव्रणहरी पुष्टिकृम्समुदीरिता ।।
तत्फलम् कफवातार्श कुष्ठारुचिर्जयेत् । गद निग्रह[16]

<u>निघण्टु रत्नाकर</u>[17]
वृक्षस्तु शल्लकी संज्ञः पुष्टकारी कषायकः ।
शीतवीर्यश्च मधुरस्तिक्तो ग्राहास्रदोषनुत् ।।

व्रणदोषं कफं वातं पित्तं चार्शम् च नाशयेत् ।
पक्वातिसारं कुष्ठं च रक्तपित्तं विनाशयेत् ।।
निर्यासोस्य मतो नाम्ना कुन्दुरुः सुज्ञभाषितः ।
कुन्दुरुः मधुरः तीक्ष्णः तिक्तो रुच्यः कदु स्मृतः ।।
स्निग्धश्चोष्णास्तथा त्वच्यो ज्वर स्वेद कफापहः ।
रक्तरुक् प्रदरं वातमलक्ष्मीं ग्रहपीडनम् ।।
रक्तातिसारं यूकां च नाशयेदपि कीर्तिः ।। निघण्टु रत्नाकर[17]

<u>शोढल</u>
शर्करारहितो मेहं वृषणस्य व्यथां हरेत् ।शोढल[18]

List of Vernacular names (local names)

Different regional names (**Indian**) of Shallaki are given below :-

Bengali:	Kundur , Luban, Salai
Hindi :	Kundur, Luban, Salai, Lohban
Gujrati:	Dhup, Gugali, Mukul, Salai
Kannada:	Chitta, Gugguludhupa, Madimara
Malayalam :	Gugguludhupam, Sambrani

Tamil :	Kundrikam
Konkani:	Visheshdhup
Telugu:	Phirangisambrani, Parangisambrani
Marathi:	Salai, , Salphullic, PahadiDhup, Vishesdhup
Oriya :	Loban
Gonda :	Bor-salla
Santal:	Salga
Deccan :	Kundur,
Bombay:	Gandh-biroza, salai

Foreign Languages:

Eng:	Indian Olibanum, Indian Frankincense
French:	Boswellie-dentelee
German:	Indischeweihrauch-baum
Arabic :	Olibanum, Kundur, Luban

Taxonomic status of shallaki [20]

Kingdom	**- Plant**
Division	**- Spermatophyta**
Sub-division	**- Dicotyledane**
Sub class	**- Polypetalae**
Series	**- Thalamyflorae***
Order (21)	**- Geraminales**
Family	**- Burseraceae**
Genus	**- Boswellia**
Species	**- serrata.Roxb.***

Etymology of the Scientific terminology:

Boswellia = After Dr. Boswel, Edinburgh, serrata= saw toothed;

B.thurifera= yielding frankinscence;

B. glabra= hairless .

Family characters of Burseraceae [21] (inscent tree family) and geographical distribution

The family is characterized by the presence of resin-ducts, compound leaves, Unisexual flowers, distinct stamens, a short single style and two ovules per locule.

Divided in to three tribes:

Tribe(i) Busereae; drupe wit endocarps of fused parts; exocarps always dehiscing by valves , Examples Bursera, Commiphora etc.

Tribe (ii) Canarieae : drupe with an endocarp of fused parts; exocarp not dehiscing by valves; Canarian .

Tribe (iii) Protieae: Drupe woth 2-5, free or adhering parts but not fused in strong endocarp; exocarp occasionally dehiscing by valves. –Protium;

There are about 17 genera and 500 species in the Burseraceae. The members of this family reach their peak of development in north Africa, tropical America, and Indian sub-continent; B. serrata Roxb. Ex.colebr., Bursera peneillat (sesse & Moc. Ex DC) Engl., Garuga pinnata Roxb. And Protium serratum (Wall.ex Colebr) Engl are trees. Several plants of the family are of value as a source of balsams, gums, resins, nuts and timber., Boswellia carterii produces " gum oilibanum" and B. frereana " african elimi".

Indian frank incense is the product of B. serrata, Mexican linaloe" of Bursera glabrfolia and B. penicillata and " dhibou" of B. gummifera. Canarium commune provides " Java almond" and C. Ovatum " pili nut". The wood of C.littorale, C.Schweinfurthii,. Dacrycodes costata, Santiria laevigata and S.tomentosa is used for carpentary and building construction. C.luronicum supplies " Maria elemi" and C.stricum " black dammar". Commipora africanum yields African bedellium, C.erythtaea "herrabol myrrh" and C.apobalsamum " Mecca balsum" Carena gum is derivied from protium carana and Brazilian elemi from P.heterophyllum. Some species of Bursera and Garuga are cultivated as ornamentals.

Listing of Geographical Distribution of Boswellia species through out the World[22]

1. Boswellia Ro. Ex Colebr- rankincense- Burseraceae
   Boswellia carteri Birdw= Boswellia sacra Flueck

2. Boswellia frereana Birdw. Names: African elemi; elemi frankincense; Yigaartree.
   Econ: Mater (Essential oil); Social (Masticatory)
   Dist: North Eastern tropical Africa.

3. Boswellia papyrifera (Delile ex. Caill) Hochst.
   Cn. Elslephant tree, Sudanese frankincense,
   Econ: Additive (flowering); Mater (essential oil); Dist; Africa

4. Boswellia sacra Flueck
   Syn: Boswellia carteri Birdw
   Ln: frankincense, olibanum tree, arbre a encenes (Fr); olibanum (Fri); Weibrauehbaum (Ger)Weihrauchpflanze (Ger); Olibano (Ital); a'rbol del incensio (Sp); Oliban (Sp);
   Econ: Mater (gum/resin); medical (folkore);
   Dist: North eastern Tropical Africa; Arabian peninsula.

5. Boswellia serrata Roxb. Syn: B. glabra.
   Cn: Indian frankincense; Indian olibanum tree; arbre a encens (Fr); kundur luban (Ind) Salai (Ind);
   Econ: Additive , (flavouring) Food (vegetable); Mater (gum/ resin, Lipids) Medic (folklore).
   Dist: Indian subcontinent;

Description & Distribution of Shallaki (Boswellia serrata Roxb.) in India :

A medium to large sized deciduous balasmiferous tree, up to 18 m in height and 2.4 m in girth (normally 1.5 m) commonly found in the dry forests from Punjab to west Bengal and peninsular India. Bark greenish grey , yellow reddish grey, fairly thick, smooth, exfoliating in thin papery flakes, resinous inside, leaves imparipinnate, 30-45 cm long; leaf lets 2-5- 6.3 cm *1.2-3.0 cm o9vate of ovate lanceolate, variable flowers small, shite,in axillary racemouse of panicles; droops 12mm long, trigonous, splitting along 3 valves, pyrenes compressed, hard, winged.

The tree is common at the foot of western Himalayas, in Rajasthan, Gujrat, Maharastra, Madhyapracesh, bihar, Orisa, Andhrapradesh and further south peninsula. The tree forms almost pure forests yielding and abundant supply timber. Large forests of this tree occur in the Khandesh

and Nagpur-wardha divisions in Mahatstra, and Khadwa-Nimar division in Madhyaprdesh, Adilabad in Andhrapradesh.

This tree prefers dry, hot exposure of rocky hills, especially the volcanic traps. On poor and shallow soil or where the ground is ferruginous, it is found pure, or nearly so. In North India, it is found on the southern slopes of the Siwalik hills, flanking the himalaya on the dry sandstone ridges associated with Acasia catechu, Anogeissus latifolia, Buchanania lanzan Spreng, Lannea caromandelica (Houtt) marill, Nyctanthus arbor-tristis Linn, Ziziphus xylopyra etc.

In Rajasthan, it occupies the drier ridges of hills on metamorphic rocks, chiefly gneiss and schists, and occurs either pure of mixed woth Lennea coromandelica, Sterculia urens Roxb. , etc, In central and peninsular India it occupies the hotter slopes and ridges, usually on trap and sometimes on gneiss and schist associated woth Acasia catechu, Anogeissus latifolia, Lannea coromandelica, S. urens, and sometimes Hardwickia binata Roxb, and *Tectona grandis* Linn. F. the tree is able to thrive and reach fair dimensions even where other species remain stunted. In its natural habitat, the absolute maximum shade temp varies from 38-49° C and the absolute minimum from –1to 7° C and the normal rainfall ranges from 50-125 Cm[23]

The tree is usually gregarious and forms comparatively open forests. It is a strong light demander. It withstands fire which is frequent in the dry tracts. It is however, frost, tender. The tree produces root suckers and pollard. Shoots is variable. The precise reason for this variation has not yet been ascertained but is probably due to geological formations, since the tree coppice well on soil consisting mainly of gneiss, Schist and quartzite, where as o must trap areas it coppices nearly. The coppice shoots, when they occur, grow rapidly flowering occurs during Jan- April, where the tree is almost leafless and fruits ripen during May, June[24]

Cultivation of Shallaki

Regeneration

Natural regeneration from seed and root suckers is usually good, even on the poorest soils, Artificial regeneration is done thorough large cuttings, over 10 cm in diam; they should be planted in the rainy season when the soil is damp. Even in drought cuttings are reported to root well[25]

Diseases and pests.

The tree is attacked by several fungi and insects. Some important fungi are Cerato stomella Sapp. And Graphivin spp. , causing blue stain or sap-stin in the logs and planks when freshly cut. Treating thelogs with sodium penta -shlorophenate (2%) and storing under cover prevent the attack. Borers and beetles- Atractocerus reversus walker, Carphoborus bosweillica stebb; coptops aedifictor fabr., Cryphalaus boswelliae stess. Megachile disuncta, platypus solidus walker, placaederus fersugineus Linn., Sinoxylon anale and Xyleborus similes Ferr etc, In fest the wood. The deterioratio mey be lessened by obtaining planks from the logs of monsoon fellings (August) and also from the logs stored for 2-3 months[26]

The tree is not readily browsed by cattle, but porcupines damage the trees by eating the bark and girdling them. The seedling are eaten by pigs[27]

Commercial uses of the Plant

A) As Timber

Timber- the tree yields a valuable Timber. The sapwood is grayish white turning grayish brown, resinous and very susceptible to fungal sap stain; the heart wood is yellowish brown to dark greenish brown, often streaked with a darker brown wavy lines. The wood is dull with rough feel, faintly scented, light to heavy (wt., 448-672 kg/ m^3), moderately strong even straight and rough grained, and medium to coarse textured. The timber is easy to saw and to work, but requires considerable care to bring it to a good surface . the heartwood takes good polish and is of good appearance, but is very refractory to treatment and difficult to season; penetration of preservative is practically nil from side or end. The large sapwood is not prone to splitting or cracking and can be treated with preservatives without difficulty. It is best to separate the sapwood and the heartwood before seasoning. Air seasoning of the timber was found to be unsatisfactory as the wood is very liable to rapid decay, mould and discoloration if left in the log , and also subject to white ant and other insect attack. Therefore it is best to convert the logs directly in the dry season after they are felled. The saw stock should be open stacked under cover to avoid mould and stain. It can be kiln seasoned satisfactorily. The seas0oned timber is fairly durable under cover. Graveyard tests on the natural durability of the untreated wood gave on average life of 23 months. However, the treatment

with preservatives makes the wood more durable. The data for the comparative suitability of the timber, expressed as a percentages of the same properties of teak are wt, 83; strength as a beem, 58; stiffness as a beam, 64; retention of shape, 96, Shear, 73 surface hardness, 5; and nail of screw holding property, 84 [28] .

The timber is suitable for manufacturing plywood, veneers, and building boards (hard boards) with requisite strength and water resistance, and of good quality. It is also used for ingerior packing, cheap furniture well construction water pipes hukkka stems sheeths for knives and swords, toys and carving and boat masts. It has been used for match splint , but its liability to fungal attack is a serious disadvantage. Ammunition boxes made from this wood are reported to be better than those of from teak wood. The wood can be used as fuel[29]

The timber is commercially exploited for paper pulp (Chemical analysis) of the wood gave the following values; cellulose 50.7; lignin, 23. 9-27.3; pentosens, 13-19.3; amd ash 1.8-2.3% . since the pulp from this wood is short fibred (av length of fiber, .88mm; diam 24 μ) an admixture with long fibred pulps is required for manufacturing news prints, and writing and printing papers with suitable strength. According to available reports almost 80% of the pu;p for newsprint manufacture is obtained from this species Braille papers, paper for wrapping and packing with satisfactory strength and agreeable colour can be prepared from the mixed pulps. It can be used in the manufacture of cellulose esters and cellulose ethers for plastic industry .

It can also be utilized for the manufacture of furfanal used for nylon. Pulps of requisite chemical purity and brightness for manufacturing tayon can be prepared from the wood in a yield of 30% by using water pre hydrolysis sulfate process, followed by multistage bleaching. The data for the yield of pulp by different methods and the suitability of the pulp for different products are presented in the table. I.

Table no.4. Yield and Suitability of Boswellia serrata wood pulp[30] (U- unbleached; B- Bleached)

Method of pu;ping	Yield %	Product
Sulphate	41.9 (B)	Paper for writing and printing.
Mechanical	85.3 (U)	Newsprint
Water prehydrolosis sulphate	30.0 (B)	Viscose rayon

B. Oleo-Gum-resin *(Kundur- Shallaki Niryasa)*

The on injury exudes an oleo gum resin known as Indian olibanum, only non coniferous source of turpentine and rosin in India. The oleo gum resin secreted from the cortex is fragrant, transparent and golden yellow and solidifies to brownish yellow tears or drops and crusts varying from pea size to walnut size. It burns readily with an agreeable odor and is chiefly used as incense. The yield varies considerably from .9-2.5 kg /tree/year. Healthy mature trees with a girth of 90 cm or more are selected for tapping. Proper tapping leaves no injurious effect on the tree. Borer damaged trees yield dome oleo resin where as dwarf, suppressed, very young or very old trees do not exude any oleo-gum-resin (Krishna & Badhwar, loc, cit, Dutt, loc, cit)

Harvesting of the Gum (collection and Preservation)

The trees are tapped by shaving off thin band of bark about 20 cm wide and 30cm long of high, at a height of 15 cm from the base of the tree. Thus initial blaze should be made to a depth of about half the thickness of the bark viz. up to .75 cm. Tapping should start from Nov-and should be stopped before monsoon. The number of blazes required depends upon the girth of the trees and initially for a tree of 90 cm girth one blaze may be made and for every increase of 50 cm girth one more blaze may be added.

Blazes may be made horizontally leaving approximately equal space between them. The lower surface of the blaze should be slightly slopping outwards to avoid lodging of the resin in the blazed pocket. The surface should be smooth and free from loose fibers. The exudate starts oozing out soon after the blazes, are made and may be collected initially after a month. Subsequent collection and freshening may be done a fortnightly of weekly intervals up to May. Thus 12 to 24 freshening may be done in all. In each freshening the lower surface of the blaze is not to be freshened but the lateral edges may be slightly scraped. The length or height of the blaze is to be increased by about 1.6 cm in fortnightly and 0.8 cm in weekly freshening every time on the upper edge. The surface already blazed are freshened may not be scrapped.

For continuous scrapping on a 3 year cycle, the bole may be divided in to three zones, each one being trapped for one year for making another horizontal row of blazes of the subsequent years should be alternating or staggering with the preceding ones. Again alternating the blazes with same zone; the blazed portions may be given complete rest for 6 years during which period

the wounds heal up and are ready for fresh tapping. This method has been developed at the forest Research Institute, Dehradun to obtain the O.D.R on a sustained basis and to avoid the risk of girdling the trees[31] The oleo-gum-resin is scrapped off and collected in a circular tray suitable placed around the trunk. It is collected in a semi solid state and the vegetable impurities are manually removed. It is then kept in baskets up to 30 days on cemented and sloping floor, whence the fluid portion containing the volatile oil is collected and used in paints and varnishes. The remaining semi solid to solid part is mainly gum rosin which is thoroughly dried and some times treated with soap stone powder to make it brittle. It is then broken into small pieces, cleaned and graded for marketing.

Grading

Generally four grades of gum rosin are known;

1. **Superfine grade** : is translucent, very light yellow in color, free from bark and other bark and other impurities;
2. **Quality I:** is brownish, less translucent and free from bark and other impurities.
3. **Quality II**: is brownish, semi translucent and may have some impurities;
4. **Quality III**: is dark brown, opaque, and with impurities. The percentage recovery of these grades varies from 10-13, 18 to 20, 20-25 and 25-30, respectively. The remaining may be left as a waste in the form of bark and impurities is also sometimes sold.

Pharmacological Considerations (Medical uses)

This section deals with the rasapanchaka vivechana as described by various authors and also tabulates the various therapeutic uses of Shallaki niryasa and twak churna.

Table no. 5.
A. Ayurvedic (Rasapanchaka of Kunduru)

Rasapanchaka		C.S	S.S	A.S/ A.H	D.N	R.N	M.N	B.N	K.N	S.N	N.R	G.N
Rasa	Madhura					+		+	+		+	
	Katu				+							
	Tikta				+	+		+	+		+	
	Kashaya	+	+			+				+		
Guna	Sheeta				+	+						
	Teekshna							+	+			
	Ushna										+	
	Snidga										+	

Veerya	Sheeta				+	+			+			
Vipaka	Katu				+				+			
Dosha karma												
	Kaphahara		+	+	+		+		+		+	+
	Pittahara						+		+		+	+
	Vatahara			+	+	+					+	+
Dhatugata karma	Medohara	+	+									
	Raktaprasadaka		+								+	
Malagata karma	Purishavirajaneeya	+		+								
	Swedapaha							+	+		+	

Table no.6

Samanya karma		C.S	S.S	A.S/ A.H	D.N	R.N	M.N	B.N	K.N	S.N	N.R	G.N
	Pustikrit										+	+
	Arocha nashak								+			+
	Twachya										+	
	Jwarahara							+	+		+	
	Graha peedahara								+		+	
	Alakshmihara								+	+		
	Sthambhi/ Grahi		+									
	Varnya		+	+								
	Vishanashaka		+	+								
	Yonidoshahara			+								

Table no. 7

Rogaghnata		C.S	S.S	A.S/ A.H	D.N	R.N	M.N	B.N	K.N	S.N	N.R	G.N
	Vrana		+			+	+		+		+	+
	Arshas (fruit)					+					+	+
	Kusta					+			+		+	+
	Pakwatisara		+				+		+		+	
	Raktitisara		+								+	
	Rakta pitta		+								+	
	Pradara				+						+	
	Yuka										+	
	Mukha roga							+	+			
	Udaramaya		+									
	Kandu, Pidaka, Kota		+	+								
	Bhagna		+									
	Upadamsha		+									
	Swasha	+										

B.From non-Ayurvedic Literature

The gum is credited with astringent stimulant, expectorant, diuretic, diaphoretic, emmenogouge, ecbolic and anti septic properties.

It is reported to be useful in ulcers, cystic breast, diarrhea and dysentery, piles and skin diseases. It is used in the preparation of ointment for sores and with butter in syphilis. The non-phenolic fraction of the oleo gum resin possesses anti tumor, sedative and analgesic activity, and is reported to produce a marked hypotensive effect on anesthetized dogs. The essential oil from olibanum showed anti bacterial activity where as the essential oil from leaves showed anti fungal activity.[32]

Clinical activity:

a. Anti inflammatory, anti arthritic

b. It is also shown to possess marked cholesterol and tri glyceride lowering activity. Clinical trial on RA patients has shown promising results. As such the possible use of *salai guggul* as ant-hyperlipidaemic and anti atherosclerotic agent is being investigated at RRL Jammu[33]

The flower and seeds are eaten. In folk medicines the dried, powdered flowers are used in colds and fevers.

Powdered Bark mixed with clarified butter is applied as a poultice on bleeding or suppurating wounds.

Other uses: The tree is valuable in afforestation of dry hills[34].

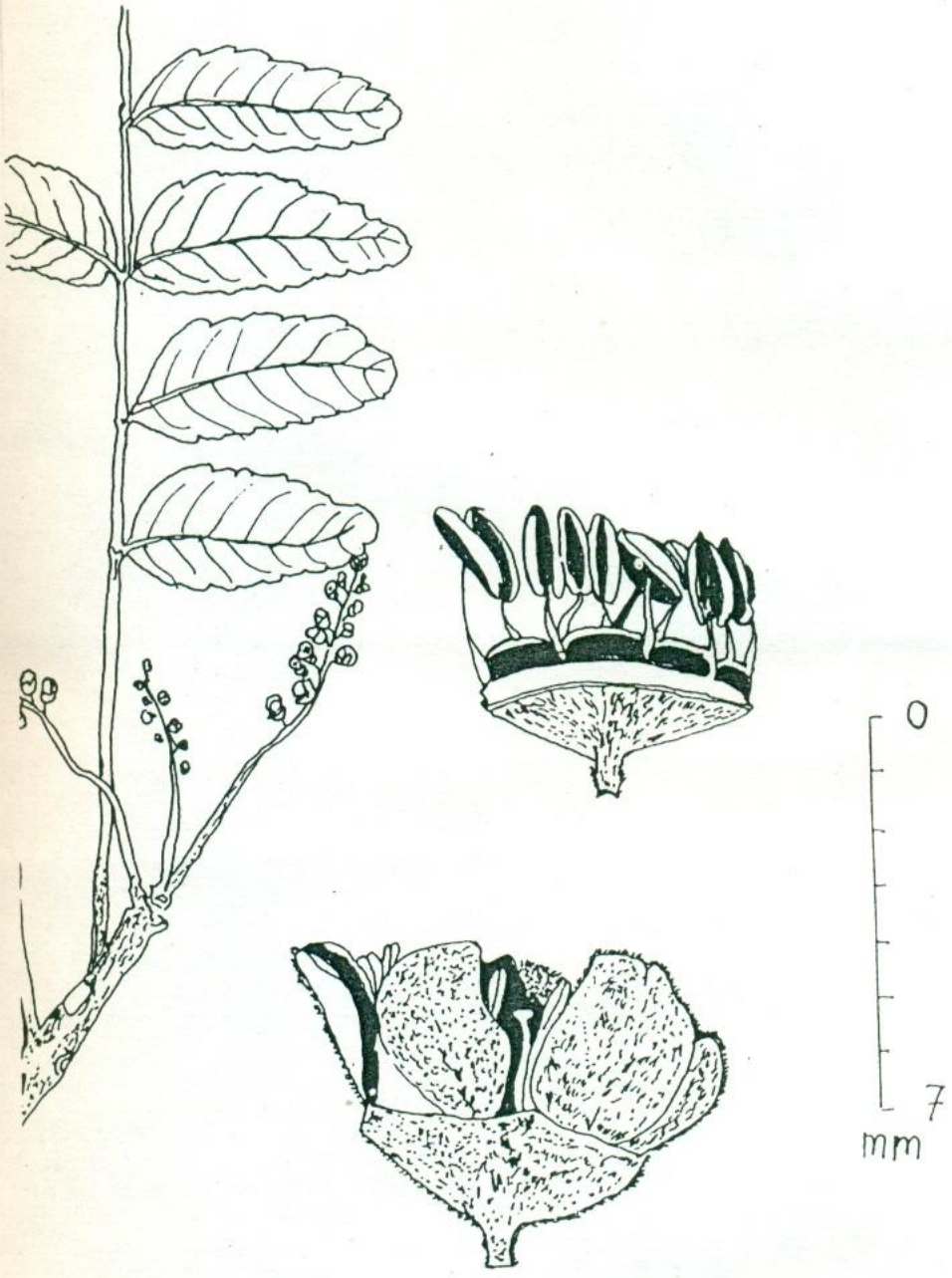
References:

1. Wiilliam Dyock , Pharmacographia indica ,Hamdard. The orgaon of the Institute of Health and Tibb research, - Hamdard national foundation, Pakistan, 1972. p. 82-84.
2. Charaka Samhita with Ayurveda Deipika commentary, Chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint,1992, p. 268,436, 437, 438.
3. Susruta Samhita with Nibandha Sangraha commentary, chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint, 1994, pp. 137-140,368
4. Ravidutta Tripathi, Astanga sangraha, Chaukamhamba Sanskrit Pratisthan, 3rd ed., 1993.
5. Pandit. Jagannatha sharma Bajpeyee, Chakradatta 4th ed. Sri Laxmi Venkateswera steam press, Kalyan Mumbai, 1959.
6. Sureshchandra Banarji, Flora and fauna in Sanskrit Literature, NyaProkash, 206, Bidhan Sarini, Calcutta 700006; p. 72
7. Krishnamurthy, K.H. Botanical identification of ayurvedic medicinal plants; a new method of Pharmacolinguistics. A preliminary account –Medicinal plants 1.MSS Information Corporation,

New York, 1974 pp. 144-157.

8. Bhavamisra, Bhavaprakasha (first part), Kashi Sanskrit series 130, 8th ed. Chaukhambha Sanskrit sansthan, PO Box no. 1139, K37/116, Gopal Mandir lane Varanasi, 1993, pp. 212, 213.
9. 10, 11. Pandit Narahari., Raja nighantu., Krishnadas Academy, Varanasi, 1882 I ed. p.255, 380, 418.
12. Dhanvantari Nighantu., Chowkhamba Orientalia,varanasi. II ed., 1998,p.112,113.
13. Ganga Vishnu Shrikrishna das., Madanapal Nighantu, Lakshminarayana Press, Kalyan, Mumbai-1954, p.93,128.
14, 15. P.V.Sharma, Ed., Kayyadeva Nighantu (Pathyapathya Vibodhaka), Chaukhambha Orientalia, Varanasi, I Ed. 1979, P.160,255.
16, 17, 18. Bapalal G. Vaidya, Nighantu Adarsh vol-I, V.Ayurveda series 54, Chaukhambha Bharati Academy, Gokul Bhawan, k. 37/109, Gopal Mandir lane, PO. Box no.1065, Varanasi-1, First ed. Pp. 266-269.
19. M. Abdul Karem, Plants in Ayurveda, FRLHT, No. 50, MSH, Layout, Anandnagar,Bangalore,, 1997, p.24.
20. S.C. Dutta, Systematic Botany, 5th Ed. New Age International Ltd. N. Delhi, 1996., P. 322.
21. S.C. Dutta, Systematic Botany, 5th Ed. New Age International Ltd. N. Delhi, 1996., P. 321
22. John. H. Weirsema, Blanca Leo'n. CRC Press, World economic plants – a standard reference- Washington DC, 1999. p. 80.
23. (Pearson & Brown, 1, 218; Mishra & Badhwar, J. Sci Industr. Res, 1948, 7B, Suppl. 117, Dutt, Indian oil Soap J, 1960-61, 26, 233; troup, I, 174, Lambie,137.
24.Troup I, 174-76,Krishnamurthy, 1956,69.
25. Troup, I, 176, 69,; Indian For, 1952, **78**, 353.
26. Purushotham, Indian for, 1951, **77**, 716; Bhasin et al, Indian for Bull, NS, Entom, No. 171 (2), 1956; 35; Stebling, 527; for ser India, 1947-48 pt I, 36; 1946-47, pt I, 175.
27. Troup, I, 175
28. Pearson & Brown, J, 218-29 Trutter, 1944, 63, 242; Khan, 9 b; Purushottam et al, Indian for, 1953, **79**, 49; J timb Dryer's preservation association India, 1956, **2**, (2); Sekhar & Gulti, Indian for Rec, NS, timb mech, 1969, 2 (1), 26
29. Narayanamurthy, Bull nat Inst, Xci India, No, 6, 1955 , 168, 180; Narayanamurthy & George, Res & Ind, 1959, **4**,, 9; Pearson & Brown , I, 220; Khan, 93; 15; 399 (Rev), 1963, 1; Quart News bull Times Dryer's Preserv. Axxoc. India, 1953, 1 (2), 20; Kapadia, J, Gujrat Res soc, 1954, 16, 6, 23
30. Singh & Mukharjee, Indian forest, 1965, **91**, 518Bhat et al , Indian for, 1952, **78**, 169; Karnik & gupta, Indian Pulp pap, 1966-67, **21**, 557; Guha et al ; bid, 1969-70, **24**, 317; 1959-60,**14**, 565.
31. Krishna Murthy & Shiva, Indian For, 1977, **103**, 466
32. Bhatnagar et al., J.Res1972, **35**, 280, ; Hartwell, ibid, 1967, **30**. 394; Shi Ji-Zhao, Dhinese med 5, 1983, **96**, 655, Kirtikar Basu, I,, 521, Ramarao 69, ; DUH, loc, cit, Dhopra et al., 1958, 595, Indian drugs pharmacy Ind, 1977, 12 (4), 17, Girgune, et al, curr sci, 1978, 47, 454Garg, Indian J. Pharm, 1974, 36-4
33. Panchnanda et al., Indian J. Pharmacol, 1981, **13**, 63, Tes & Ind, 1982, 27, 304; Singh et al, ibid, 1981. 13, 97
34. Ramarao, 70, Kapoor et al., Sci & Sult, 1975, **41**, 330, Dixit and Panday, Inst J. Crude Drug Res, 1984, 22, 47; Karnik et al, Indian J. Crue Drug res, 1984, 22, 47,: Karnic et al., Indian For, 1970, **96**, 314, Mukharji et al Indian J. pharm, 1970, **32**, 48, Bhatnagar et al loc, cit.

Twig of Shallaki (Boswellia serrata Roxb.,)



Boswellia serrata Roxb ex Coleb.
a - twig ; b - bud ; c - flower ; d - fruit

Shallaki –Entire plant & Habitat

Twig of Shallaki [foliage]

Oleo –gum –resin [Fresh from shaving]

Stem of shallaki – External appearance

Fruits of Shallaki

Materials and methods

part II

8. Materials and Methods Part -II

Part-II. Botanical Standardization
Macroscopic & Microscopic Characters

This chapter covers macroscopic and microscopic features of the Shallaki (Boswellia serrata Roxb.) bark and the oleo-gum- resin. The standard pharmacognostic nomenclature has been used to specify the details of the observations made during the study. For studying the features of the bark both the immature plant stem (twigs) as well as the mature bark are taken into consideration.

For the macroscopic study both the morphological appearance of the plant on fresh cuttings, the dried ones and the powdered forms are taken in to consideration.

Gum collected from various sources including the fresh oozing, dried raw material, and the powdered forms of the plant are documented and every effort has been made to observe the similarities and dissimilarities between the various samples and the conclusions are drawn up on them. The findings are:

A. Characteristic features of the Bark:

Fresh samples:

Procedure (naked eye examination; and examination with a hand held lens)

Bark greenish (due to presence of Chloroplasts) gray, yellow reddish gray, fairly thick, smooth, exfoliating in thin papery flakes, resinous inside (External appearance)

On cutting the bark appears brick red in color has a characteristic smell (turpentine/lemony).

On drying the bark surface will not loose the greenish color and breaks very easily [with waxy fracture].

The characters of the Bark powder:

It is brick red in color, and has the characteristic smell.

On soaking and triturating with water it makes the color of the water very dark red in color.

Microscopic characters of Bark powder:

Procedure:

For studying the microscopic characters of powder 85 no. sieve is used to get the fine powder .

1. The powder is placed first on the Slide with glycerin and a cover slip is paced to secure the preparation the observed under the microscope under(1085-50X, 40 *5 -200X) magnification.

2. Powder is taken in sufficient amount in Chloral-hydrate solution on a slide and covered with a cover slip, warmed over a low flame for a short time. This procedure is essential to separate the different constituents of the material

3. The specimen Prepared for examination by placing a little amount of powder on a slide, 1-2 drops of *phloroglucinol* and a drop of concentrated *hydrochloric acid*, cover slip, draw off the liquid from one side of the slide with filter paper, and then 1-2 drops of *chloral-hydrate solution* is applied from the other side of the slide[1]

Observations:

The sample shows Bark cells[bast tissue, companion cells, , crystals, compactly packed brownish colored pigment cell clusters, parenchyma cells Lignified elements, and sieve tubes are seen.

Microscopic characters (Sections of Shallaki)

The transverse and longitudinal sections of the young stem as well as the old stem are made from the fresh cuttings and the dried specimens also.

For softening the dried samples two types of procedures are applied.:

1. The cuttings are soaked in a solution water and glycerol for about three days, and the sections are prepared afterwards.
2. 1-2 CM long pieces with a width of 0.5-1 are taken in a test tube and boiled for 3 min, then the samples are straightened with a scalpel and placed in wax mould. Then the sections are prepared using the razor/micro-tome[2].

a. Young stem:

In transverse section the rectangular cork cells originating from sub epidermis are clear. They are arranged in a super imposed rows and fit together.

Vascular bundles consist of a closed cylinder. Phloem cells contain the calcium oxalate crystals . In this region 29-36 canals of schizo-lysigenous nature are noted; they are partially filled with resinous substance.

Cambium contains three to five layers of tangentially elongated cells, filled with starch grains.

In the xylem region vessels , fibers, tracheids and parenchyma are quite distinct. Pith is heterogeneous in nature and consist of thick-walled parenchymatous cells with intercellular spaces. Calcium oxalate crystals Oil globules are abundantly noted in pith region.

b. Mature stem

The cork zone is composed of many layers of tangentially elongated some what rectangular cells, arranged in radial rows with slightly wavy and thick walls. The crushed cells are also abundantly present. It measures 12.2-29.7-49.5-71.7 μ* 3.2-13.2-24.1-32.0μ. The meristmatic cells which originate from the cortical parenchyma are also visible.

Cortex zone contains thin walled parenchymatous cells. Small oil globules are also noted. It measures 14.2-41.9-62.3μ*12.2-9-28.7-40.9μ .

Xylem and phloem consist of closed cylinder traversed by 1-*3 celled wide medullary rays. In the lower part of the xylem some rounded oil globules are also present with in the cells. Cambium cells contain three to five layers of crushed cells .

The shizo lysigenous canals are embedded in the phloem region. The canals originate shizogenously but in future development the epithelial cells break down forming an resinous secretion and the cavity continues to increase in size forming a broad schizo-lysigenous cavity. The canals develop near the cambium and shift towards the cortex as soon as the new canals are originated. Canals measure, 24.4-45.5-63.0μ*44.9-62-90.4μ..

Pith is heterogeneous and is formed of rounded somewhat thick walled cells of parenchyma. It contains rounded oil globules which measure 3.2-17.8μin diameter.

In the tangential sections the vessels are long broad and marked with clearly defined piths. Wood parenchyma varying from scanty to vesci-centric and occasionally with some diffuse cells. Wood fibers are also elongated, thick walled and tapering on both ends. The medullary ray bundles vary in width from one to three cells. The end of the bundles are always one celled in width.

Secondary medullary rays are noted accompanying the primary medullary rays. They are ten to fifteen cells in width. Cells of secondary rays have the thickenings. In the radial sections, the height of the medullary rays varies depending on the portion, the sections have passed. The simple pits on the vessels are clearly visible. The fibers and parenchyma are alike[3].

Ultra-microscopic details or Boswellia serrata Roxb. Stem and the Oleo-gum-resin duct (Shozolysigenous ducts.)

Oleo-gum-resin ducts are present in primary and Secondary phloem. The Duct lumen forms and enlarged apoplastic space surrounded by epithelial cells. The epithelial cells are rich in dictyosomes, lipid bodies, mitochondria with dilated cristae, multi vesicular bodies, osmiophilic materials, plastids and vesicles. Plastids have poorly developed internal membranes. Dictyosomes and plastids are possible sites of resin synthesis. The gun component of the exudate is formed in dictysomes and from the outer layers of the inner tangential wall (wall facing the duct lumen). This wall is replenished from inside by the activity of dictyosomes.

The secretary materials are transported to the apoplast by granulocrine and eccrine secretion. They migrate through the loose micro-fibrils of the inner tangential wall into the duct lumen. Rarely, epithelial cells of young ducts have rudimentary plasmodesmata on the inner tangential wall, which may be channels for passage of secretary materials into the duct lumen[4].

B. Macroscopic/ Microscopic Characters of oleo-gum-resins powder:

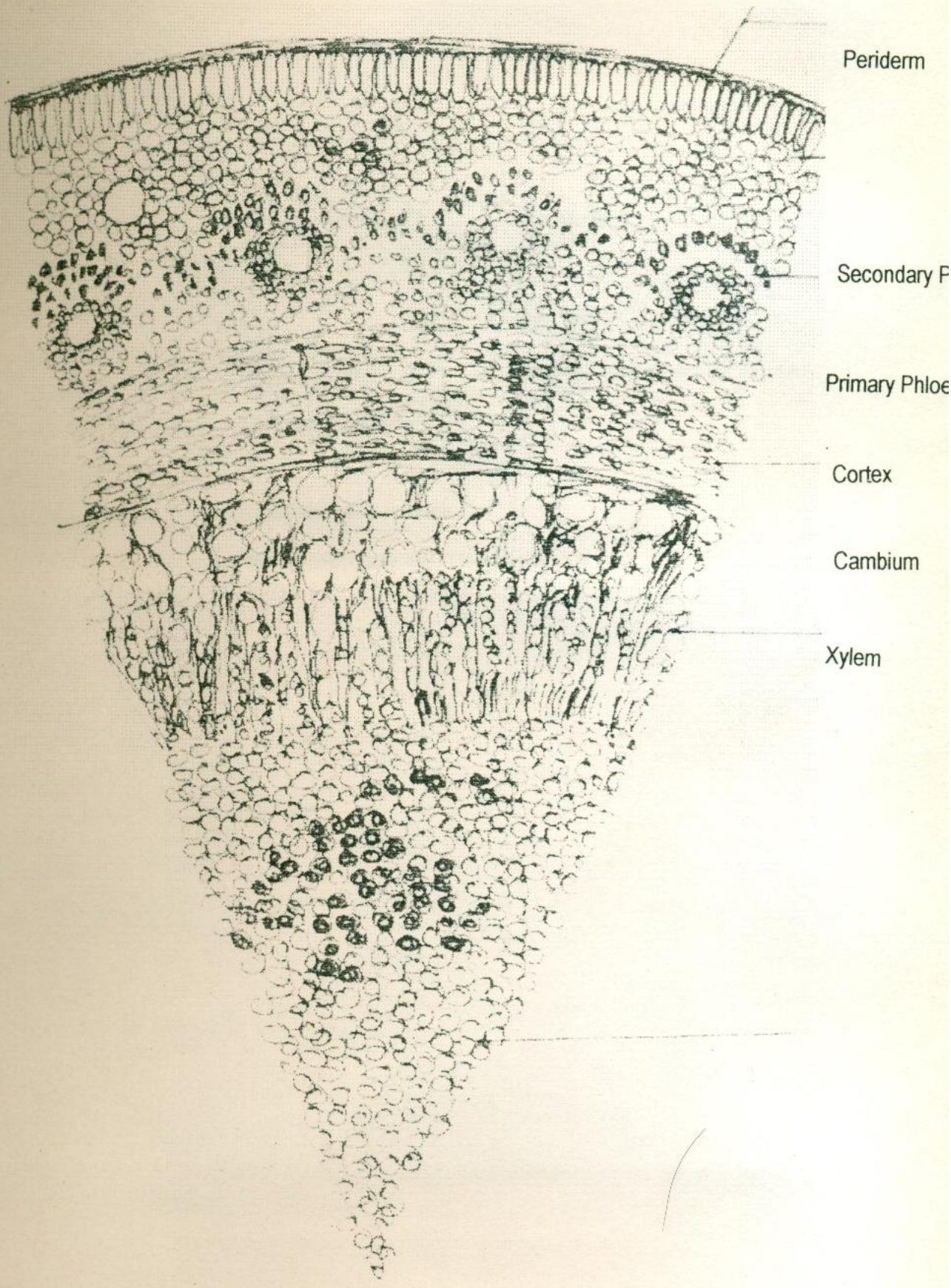
Occurs in small and large tears, dark brown to cream colour. Soft, gum like texture, Characteristic turpentine like Agreeable on burning. Soluble in warm water with milk like appearance [characteristic feature]

Alcohol dissolves resin, leaving angular non crystalline particles of gum and fragments of bark. Powder when treated with % NaOH and 5% FeCl3 no change in color occur either it gives any stain when pressed between filter paper.

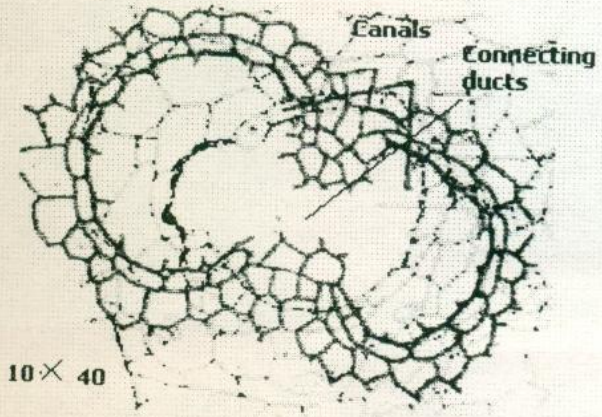
Gum shows irregular [angular] shaped transparent gum, opaque ,blackish[resinous material], plant residue is seen.

Ref:

1. A. The Ayurvedic Pharmacopoeia of India, vol-1, part-II, Dept. of ISM, Ministry of Health and Family Welfare, Govt. of India, 1999, appedix-1, pp. 183.
2. b. Anonymous., Mannual for Quality control of Herbal Drugs, National Botanical Research Institute, Lucknow-1, 2001. Chapters 2, 3.
3. Ibid.,
4. Dennish-TJ; Billore-KV; Mishra-KP; Pharmacognostic study on gum oleo-resin of Boswella serrata Roxb. Dept. of Chemistry . , Osmania Univ . , Hyderabad, India; Bulletin-of Medico-ethno-Botanical-Research; 1981, 1:3, 253-260.
5. Nair-MNB; Subrahmanyam-SV . , Ultrastructure of epothelial cells of oleo-gumresin secretion in Boswellia serrata (Burseraceae). , Faculty of forestry, University Putra Malaysia, 43400 UPM Serdang Selangor, Malaysia; IAWA – Journal. 1998, 19:4, 415-427;
6. A.Fahn, Plant anatomy, 4th ed., Pergamon Press., 1990 p. 79, 134, 185, 309.



Transverse Section of young stem of Shallaki (Boswellia serrata Roxb.,)



Shizolysigenous ducts

canal surrounded by the Phloem cells - 10 × 40

Kundur – sample from Hyerabad A

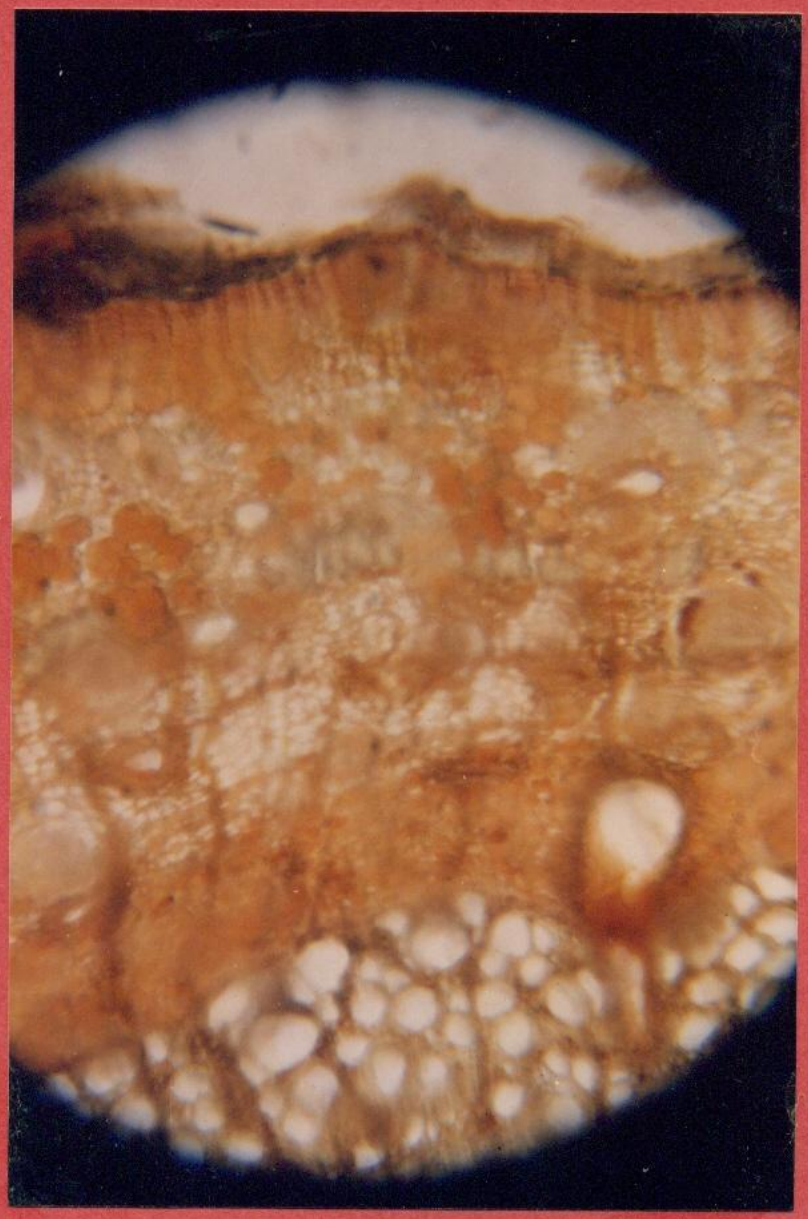
Kundur- sample from Jaipur Market

Kundur- Natural & Market samples

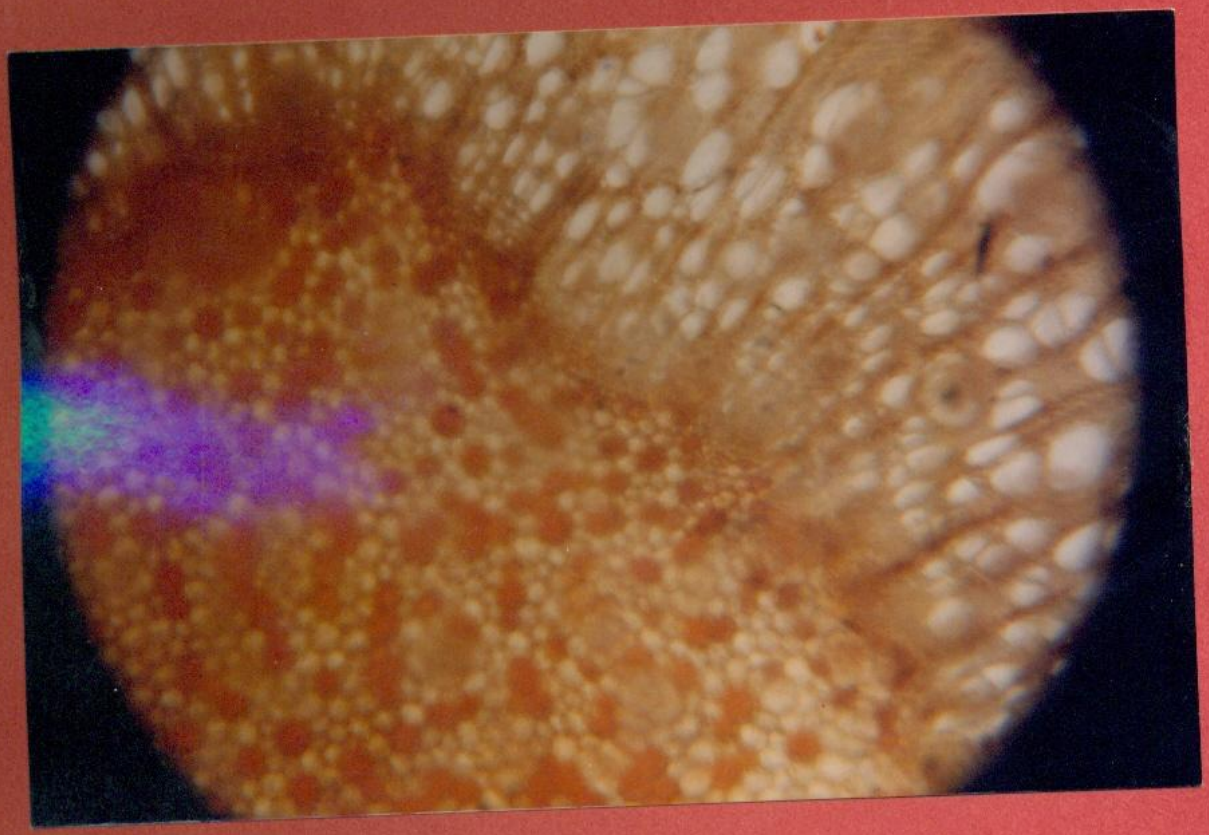
Spurious drug- Hyd B-sample

Shallaki Bark – Khandwa forest

Shallaki bark and bark powder



T.S of Stem [B. serrata Roxb.]- Outer region



T.S of Stem – v. bundles and Pith region

Materials and Methods

Part III

9. Materials and Methods-Part III

Determination of Quantitative Data

DETERMINATION OF QUANTITATIVE DATA OF DRUGS TEST [1]

Preparation of The test Sample

Required amounts of samples are collected by quartering, taking care to see that the portion represents the gross sample. The ground samples of powdered drugs are passed through the 85 no. sieve to obtain the fine powder.

A. FOREIGN MATTER

Drugs should be free from moulds, insects, animal fecal matter and other contamination such as soil, stones and extraneous material.

Foreign matter is material consisting of any or all of the following: -

(1) In particular, parts of the organ or organs from which the drug is derived other than the parts named in the definition or for which a limit is prescribed in the individual monograph.

(2) Any organ or part of organ, other than those named in the definition and description. The amount of foreign matter shall not be more than the percentage prescribed in the monograph.

1.DETERMINATION OF FOREIGN MATTER

100g. of the drug sampled is taken and spread as a thin layer. The foreign matter is examined by examination [inspection] with unaided eye / and the use of a lens (6X). Separated material is weighed and the percentage is calculated.

Table No. 1. Foreign matter of the bark sample

S.No.	Name of the place collected	Weight of the foreign matter collected	Percentage(%)
1.	Thamsa forest, Nanded Dist.	2g	2
2.	Keesara gutta,Hyderabad, A.P	1.89g	1.89
3.	Khandwa, Madhya pradesh	3g	3

Table No. 2. Foreign matter of The Oleo-gum-resin sample

S.No.	Name of the place collected	Weight of the foreign	Percentage(%)
1.	Thamsa forest, Nanded Dist.	1g	1
2.	Hyderabad,A. (Kundur)	5g	5
3.	Hyderabad,B	1g	1
4.	Jaipur market	3g	3
5.	Himalaya drug co.	Nil	Material in capsules.

2. DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING)

About 10 g of drug (without preliminary drying) is placed after accurately weighing (accurately weighed to within 0.01 g) it in a tarred evaporating dish.

After placing the above said amount of the drugs in the tared evaporating dish dry at 105° for 5 hours, and weighed. The drying and weighing at one-hour interval is continued until difference between two successive weighing corresponds to more than 0.25 per cent. When constant weight is reached in two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, showed not more than 0.01 g difference.

Table No. 3 Moisture content of the Bark

S.No	Name of the place	Initial weight	Weight afterwards	Loss	pecentage
1.	Thamsa, Nanded dist.	10.0013 g	8.5013	1.5001	15
2.	Hyderabad	10.0018	8.2003	1.8015	18.011
3.	Khandwa	10.0214	8.4212	1.6002	15.697

Table no. 4. Moisture content of the Kundur.

S.No	Name of the place	Initial weight	Weight afterwards	Loss	percentage
1.	Thamsa, Nanded dist.	10.0215	8.9192	1.1023	10.99
2.	Hyderabad A	10.1003	8.9949	1.1054	10.94
3.	Hyd. B	10.1058	9.6963	0.4095	4.0521
4.	Jaipur	10.2314	9.1099	1.1215	10.96
5.	Himalaya Drug Co.	10.1245	9.0236	1.1009	10.87

3. DETERMINATION OF TOTAL ASH

about 1 g of accurately weighed, ground drug is incinerated in a tared platinum or silica dish at a temperature not exceeding 450° until free carbon, cooled and weighed. The percentage of ash is calculated with reference to the air-dried drug. (weight of the ash will be the difference between the Initial weight of the crucible and after the incineration;)

Table no.5. Total Ash of Bark Powder

S.no	Place of collection	S.No	Weigh of the crucible (before)-W1	Weigh of the crucible (After)-W2	Weight of the Ash=w2-w1	Ash in %
1.	Nanded	i.	10.2368	10.3218	0.085	8.5
		ii.	10.2368	10.3214	0.0846	8.4
		iii.	10.2370	10.3220	0.085	8.5
		Average				8.46
2.	Hyderabad	i.	11.7965	11.8755	0.0792	7.9
		ii.	11.7968	11.8748	0.0780	7.8
		iii.	11.7966	11.8786	0.0820	8.2
		Average				7.9
3.	Khandwa, M.P	i.	10.2370	10.3221	0.086	8.6
		ii.	10.2368	10.3215	0.0847	8.4
		iii.	10.2370	10.3218	0.0846	8.4
		Average				8.4

Table no.6 Total Ash of Kundur (Shallaki niryas)

S.No	Place of collection	S.No.	Weigh of the crucible (before)-W1	Weigh of the crucible (after)-W2	Weight of the Ash=w2-w1	Ash in %
1.	Nanded (collected from forest)	i.	10.2356	10.2570	0.0214	2.14
		ii.	10.2359	10.2568	0.0209	2.09
		iii.	10.2360	10.2565	0.0205	2.05
		Average				2.09
2.	Hyderabad [A]	i.	10.2358	10.2570	0.0212	2.12
		ii.	10.2360	10.2569	0.0209	2.09
		iii.	10.2361	10.2566	0.0205	2.05
		Average				2.0
3.	Hyderabad [B]	i.	10.2360	10.2919	0.0559	5.59
	(Gold colored sample	ii.	10.2358	10.2921	0.0563	5.63
		iii	10.2362	10.2918	0.0556	5.56
		Average				5.59
4.	Jaipur Market	i.	11.7965	11.8179	0.0214	2.14
		ii.	11.7964	11.8175	0.0211	2.11
		iii.	11.7966	11.8176	0.021	2.1

		Average				2.11
5.	Sample of Himalaya drug Co.	i.	10.2356	10.2571	0.0215	2.15
		ii.	10.2358	10.2573	0.0215	2.15
		iii.	10.2360	10.2568	0.0208	2.08
		Average				2.12%

4. DETERMINATION OF ACID INSOLUBLE ASH

The ash obtained as above method is boiled for 5 minutes with 25 ml *of dilute hydrochloric acid;*The residue(the insoluble) is collected matter in a crucible, on an ash less filter paper, washed with hot water and ignited to constant weight, then the percentage of acid-insoluble ash is calculated with reference to the air dried drug.

Table no.7 Acid insoluble Ash of Bark Powder

S.No	Place of collection	S.No.	Weigh of the crucible (before)-W1	Weigh of the crucible (After)-W2	Weight of the Ash=w2-w1	Ash in %
1.	Nanded	i.	10.2368	10.2620	0.0252	2.5
		ii.	10.2368	10.2318	0.0250	2.5
		iii.	10.2365	10.2628	0.0263	2.6
		Average				2.5
2.	Hyderabad	i.	10.2350	10.2620	0.0270	2.7
		ii.	10.2368	10.2318	0.0250	2.5
		iii.	10.2368	10.2621	0.050	2.5
		Average				2.5
3.	Khandwa, M.P	i.	11.7966	11.8229	0.0263	2.6
		ii.	11.7965	11.8230	0.0263	2.6
		iii.	11.7965	11.8230	0.0265	2.6
		Average				2.6
	Final value					2.5%

Table no.8 Acid insoluble Ash of Kundur (Shallaki niryas)

S.No	Place of collection	S.No.	Weigh of the crucible (before)-W1	Weigh of the crucible (after)-W2	Weight of the Ash=w2-w1	Ash in %
1.	Nanded (Collected from Forest)	i.	10.2360	10.2525	0.0145	1.4
		ii.	10.2361	10.2501	0.014	1.4
		iii.	10.2362	10.2500	0.0138	1.38
		Average				1.38
2.	Hyderabad [A]	i.	11.7965	11.8115	0.015	1.5
		ii.	11.7966	11.8114	0.0148	1.48
		iii.	11.7965	11.8114	0.0149	1.49

Table no.10 Water insoluble Ash of Kundur (Shallaki niryas)

S.No	Place of collection	S.No.	Weigh of the crucible (before)-W1	Weigh of the crucible (after)-W2	Weight of the Ash=w2-w1	Ash in %
1.	Nanded	i.	11.7965	11.8137	0.0172	1.72
		ii.	11.7966	11.8138	0.0172	1.72
		iii.	11.7964	11.8136	0.0172	1.72
		Average				1.72
2.	Hyderabad [A]	i.	11.7965	11.8136	0.0171	1.71
		ii.	11.7966	11.8140	0.0174	1.74
		iii.	11.7967	11.8138	0.0178	1.78
		Average				1.7
3.	Hyderabad [B]	i.	10.2360	10.2542	0.0182	1.82
	(Gold colored sample	ii.	10.2362	10.2542	0.0179	1.79
		iii.	10.2361	10.2542	0.0181	1.81
		Average				1.8
4.	Jaipur Market	i.	10.2362	10.2534	0.0170	1.7
		ii.	10.2361	10.2532	0.0171	1.71
		iii.	10.2360	10.2534	0.0174	1.74
		Average				1.72
5.	Sample of Himalaya drug Co.	i.	10.2360	10.2534	0.0174	1.74
		ii.	10.2362	10.2532	0.017	1.7
		iii.	10.2361	10.2534	0.0172	1.73
		Average				1.71

B. Extractive values

6. DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE

Macerated 2. 5 g of the air dried drug, coarsely powdered, with 50 ml of Alcohol of the specified strength(60%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Then Filtered rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105° to constant weight and weigh. Then the percentage of alcohol-soluble extractive is calculated with reference to the air-dried drug.

Note: Extractive values are performed using alcohol, water and petroleum ether as extracts. The final values are calculated approximating the values to 5g and 100ml ; the original values taken are 2.5 g and 50 g of solvent. Hence the values are multiplied by 40 to obtain final value.

Table 11- Alcohol soluble extractive of bark powder

S.No.	Source	Weight of the Petri-dish before [tarred](W_1) in grams	Weight after collecting and evaporating the extract (Constant) (W_2) In grams	W_1-W_2=W	W Multiplied by 40 Value in %
1.	Thomsa forest, Nanded ,Maharastra	51.4252	51.8875	0.4632	18.492
2.	Keesaragutta, Hyderabad, AP	50.9645	51.4343	0.4698	18.792
3.	Khandwa, M.P	42.2354	42.6916	0.4562	18.248

Table no.12- Alcohol soluble extractive of gum-oleo- resin powder

S.No.	Source	Weight of the Petri-dish before [tarred](W_1) in grams	Weight after collecting and evaporating the extract (Constant) (W_2) In grams	W_1-W_2=W	W Multiplied by 40 Value in %
1.	Thomsa forest, Nanded ,Maharastra	35.2716	36.6928	1.4212	56.848
2.	Hyderabad A	42.9715	44.4217	1.4502	58.008
3.	Hyderabad B.	57.9480	59.5494	1.6014	64.056**
4.	Jalpur	35.2817	36.7319	1.45042	58.008
5.	Himalaya D.Co.	51.4252	52.8756	1.4504	58.016

7. DETERMINATION OF WATER SOLUBLE EXTRACTIVE

The same Procedure is followed for the determination of Alcohol-soluble extractive, using *chloroform* •*water* instead of ethanol.[2.5 g of sample and 50 ml of the solvent].

Table 13- Water soluble extractive of bark powder

S.No.	Source	Weight of the Petri-dish before [tarred](W_1) in grams	Weight after collecting and evaporating the extract (Constant) (W_2) In grams	W_1-W_2=W	W Multiplied by 40 Value in %
1.	Nanded	61.8600	62.2995	0.4395	17.58
2.	Hyd	44.6390	45.277	0.4380	17.52
3.	Khandwa	42.9701	43.4121	0.4420	17.68

Table 14- Water soluble extractive of oleo-gum-resin powder

S.No.	Source	Weight of the Petri-dish before [tarred](W_1) g.	Weight after collecting and evaporating the extract (Constant) (W_2)In g	W_1-W_2=W	W Multiplied by 40 Value in %
1.	Nanded	49.2300	49.5872	0.3572	14.288
2.	Hyd A	42.9712	43.3346	0.3634	14.3560
3.	Hyd B	Not soluble in water.			
4.	Jaipur	44.8390	45.1928	0.3538	14.152
5.	Himalaya D.co.	51.8602	52.1824	0.3434	13.736

8. Ether soluble extractive (Fixed oil content –non volatile oil fraction)

Table 15- Ether soluble extractive of bark powder

S.No	Source	Weight of the Petri-dish before [tarred](W_1) in g	Weight after collecting and evaporating the extract (Constant) (W_2)In grams	W_1-W_2=W	W Multiplied by 40 Value in %
1.	Nanded	61.8600	62.2995	0.4395	17.58
2.	Hyd	44.9162	45.3582	0.4420	17.68
3.	Khandwa	49.5872	50.0404	0.4532	18.128

Table 16- ether soluble extractive of bark powder

S.No.	Source	Weight of the Petri-dish before [tarred](W_1) in g	Weight after collecting and evaporating the extract (Constant) (W_2)In grams	W_1-W_2-W	W Multiplied by 40 Value in %
1.	Nanded	42.9712	49.5872	0.3572	14.286
2.	Hyd A	35.2716	35.6205	0.3489	13.956
3.	Hyd B	44.3870	44.9160	0.1161	4.644
4.	Jaipur	61.8600	62.215	0.3550	14.2
5.	Himalaya D.Co	44.3870	44.755	0.3680	14.72

9. Determination of Volatile oil content:

The determination of volatile oil in a drug is made by distilling the drug with a mixture of *water* and *glycerin*, collecting the distillate in a in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile is expressed as a percentage v/w.

Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml *of glycerin* and 175 ml of -*water* in the one litter distilling flask, and a pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into graduated receiver, keeping the tap open until the water overflows, any air bubbles in the rubber tubing are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences.

The distillation is continued at a rate which keeps the lower end of the condenser cool The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap is opened and the tube lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the tap is closed and the volume is read.

The tube is then raised till the level of water in it is above the level of when the tap is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug.

Table no. 17. Volatile oil content of the Bark powder

S.no	Place of collection	Weight of the material	Volume	% V/W
1.	Nanded dist.	50g	1ml	2
2.	Hyd	50g	1.5ml	3
3.	Khandwa	50g	1ml	2

Table no. 18. Volatile oil content of the Gum

S.no	Place of collection	Weight	Volume	% V/W
1.	Nanded	50g	4ml	8
2.	Hyd A	50g	4.5ml	9
3.	Hyd B	50g	1ml	2
4.	Jaipur	50g	5ml	10
5.	H. D.co.	50	4ml	8

10. Thin Layer chromatography of Shallaki Bark and Shallaki Niryasa.

TLC is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through absorption and a mobile phase in the form of liquid. By this we can separate individual compounds from a mixture. Semi quantitative identification of compounds can be made by observing size, integrity, magnitude an RF value of separated spots.

Thin Layer chromatography study of the three samples (one bark sample, one sample of Shallaki niryasa and the gold colored sample sold in the name of Shallaki at Hyderabad market) were carried out by using following conditions:

- Adsorption layer: Silica gel GF 254
- Samples : Alcohol and Ethanol extracts of the Samples
- Solvent systems (mentioned in the tables)
- Detection by : 1. Under long wave UV radiation
  2. Exposure to iodine vapor.

Table no 19. TLC values.

S.No	Sample	Solvent	Distance traveled by the sample	Spots	Distance of spots	Rf value
1.	Kundur – alcohol extract	Petroleum ether 40/60- 100%	11cm	a.	2cm	0.18
				b.	3cm	0.27
				c.	5cm	0.45
2.	------- do ------	Acetone: Chloroform: water (70: 30: 2)	13.5cm	a.	12cm	0.88
3.	Kundur- Petroleum ether extract 1.	Petroleum ether 40/60- 100%	12cm	a.	8.5	0.708
				b.	10	0.83
4.	Do [plate no2]	------- do --------	12.5cm	a.	8	0.64

				b.	9.5	0.76
5.	Kundur – alcohol extract	Petroleum ether 40/60	14cm	c. a.	11 12.5	0.91 0.89
6.	Kundur Ether extract no. 1	Chloroform 100%	13cm	a.	12	0.92
7.	----Do--- no. 2	-------do--------	12cm	a.	9	0.75
				b.	11.5	0.95
8.	Kundur alcohol extract	-------do--------	12	a.	8	0.66
9.	Bark alcohol extract	Ether:Chloroform 30: 40	13.5	a.	12	0.88
10	Bark petr.ether extract	Petroleum ether 40/60	13	a.	8	0.61

Impression:

1. a. The alcoholic extract of Kundur (gum oleo resin of shallaki) has developed spots with Rf values 0.18,0.27,0.45, 0.85 with petroleum ether as solvent.
   b. With acetone: chloroform: water (70:30: 2) as solvent – 0.88;
   c, Chloroform as solvent –0.66; (total 6 spots)
2. The Petroleum ether extract of the Kundur (gum oleo resin of shallaki) with
   a. Petroleum ether as solvent developed – 0.64,0.70,0.76,0.83,0.91
   b. Chloroform as solvent developed – 0.75,0.92, 0.95 (8 spots)
3. The alcohol extractive of the Bark developed
   a. with Ether:Chloroform 30: 40 as solvent – 0.88,
4. Petroleum ether extractive and Ether as solvent – 0.61 (2 spots)
5. the golden coloured sample (later confirmed as the residue of colophohy) has not developed any type of chromatogram in an any extractive and type of solvent.

11. UV- VIS spectrophotometric analysis[3]:

Spectrophotometric analysis involves the measurement of the ability of the dissolved substance to absorb electromagnetic radiation of definite and narrow wavelength ranges. These absorption are measured at wave lengths that are generally characteristic of the chemical composition of the dissolved absorbing substance. Radiant energy waves ranging from 200 to 380 nm is the UV region and from 380 to 750 nm is visible region. The UV Visible spectra of the substance generally do not have a high degree of specificity but they are suitable for quantitative assays for many substances and useful as additional means of identification.

An attempt has been made to evaluate whether UV visible spectrophotometry can be utilized for the comparison and analysis of the samples.

For This purpose the accurately weighed quantity of the samples were taken separately in two different glass flasks and shaken well, tightly closed . the flask and the material are allowed to stand over night. Then filtered. This filtrate was taken for UV-Visible Spectrophotometer analysis. The spectra recovered in recording after suitable dilution are recorded. The data is as follows:

1. Spectra of Kundur (oleo –gum resin) Petroleum extract has shown peak absorbency at 680 nm and the other relevent points are mentioned in the (fig no 1.)
2. Spectra of Percentage If transmittance of the Petroleum ether extract of Shallaki bark has shown a peak at 280nm. (fig no 2.)
3. Kundur – alcohol extract does not show any significant absorption in UV spectra (fig. no 3). It shows peak pick at 474.4 nm in vis spectra. (fig no. 4)
4. Bark alcohol extract in UV- spectra shows peak absorption at 205.6 nm followed by other points at 215.2, 256.4, 248.4, 306.8 nm. (fig. No. 5)
5. Bark alcohol extract shows peak pick at 500nm in the vis. Spectra (fig. No. 6.)

Chemical composition of Boswellia serrata Roxb. Gum.

The Oleo gum resin is a complex mixture of terpenoids and sugars. In broad terms the chemical constituents may be divided in to three main groups;

I. **Volatile oil or lower terpenoids**;

Steam distillation of fresh gum resin yields up t o 10-16 % essential oil with characteristic odour. Phydico chemical characteristics of the volatile oil depends up on the source materials. The monoterpenes, identified in the oil include α– pinene, β- pinene ,α - thujene, δ- limonene, p-cymene, cadinene, geraniol, elemol, terpeneol, methyl chavicol and phallandrene[4].

II. **Higher Terpenoids.**

Higher terpenoids constitute the major fraction (25-35 %) of the oleo gum resin. A good amount of work has been carried out on the chemistry of higher terpenoids since the first isolation of boswellic acid in 1898 by Tschirch et al[5].

Since then number of chemists have worked on the structure elucidation of β–boswellic acid . Some eminent names include Ruzicka, Ferdinado, Simpson, Biham et al. Graham Allen provided the chemical evidence for disclosing the Stereo identity of hydroxyl

and carboxyl functions and configuration at c-5, c-8, c-10,c-13 and c-17. he finally established that configuration of hydroxy and carboxyl groups in β– boswellic acid is axial. Structure of methyl ester of acetyl- β- boswellic acid has also been confirmed by single orbital X-ray studies recently. 24. β– boswellic acid has some characteristic features in its structure. It belongs to ursane group of triterpnoids with C-3 hydroxy function oriented axially which is not very common in this group. Moreover there is a carboxylic function at C-4 which is also oriented axially making ring 'A' little strained. The broad angle between C-5, C-4 and C-24 is 115-9^0 .The axially oriented hydroxy and carbohydroxyl groups make the molecule more susceptiable to certain chemical transformations e.g. oxidation to 3-keto derivative invariably lead to decarboxylation.

Besides - boswellic acid several other triterpinoids have also been isolated from the gum resin, these compounds include - amyrins, 11- keto - boswellic acid, 3'-bydroxy urs-9, 11-dien-24-oic acid . Tetracyclic triterpenoic acids have also been reported by Pardhy et al[6-7]

These compounds are 3'-hydroxy-triurcall-8, 24-dien-21-oic acid, 3 keto-tirucall-8, 24-dien-21-oic acid (fig). Besides these authors have also reported the isolation of a new diterpene alcohol **serratol**.

More recently mahajan et al 28 have reported the isolation and structure elucidation of two new triterpenoids from acidic and uatural fractions of the gum extract, the structure of these compounds are 21-3'-dihydroxy Urs-12-ene-24-oic acid and urs-12-ene-3'-24 diol.[8]

III. Carbohydrates

Carbohydrates or sugars also constitute the major fraction (45-60%) of the gum resin. Preliminary examination indicated the presence of disacharides along with oligo and polysaccharides. Although not much work has been carried out in the gum resin come earlier work reported by Malandkar 30 et al edentified the acid hydrolyzed products as arabinose, xyloseand galactose[9]

Methods of isolation[10]

Extensive pharmacology of different extracts of the gum resin has led to the identification of the active fraction .this fraction constitute mainly the triterpenoic acids which include both pentacyclic and tetracyclic triterpenoic acids. For the extraction and

separation of triterpenoic acid fraction (BA) from the gum resin following methods may be adapted

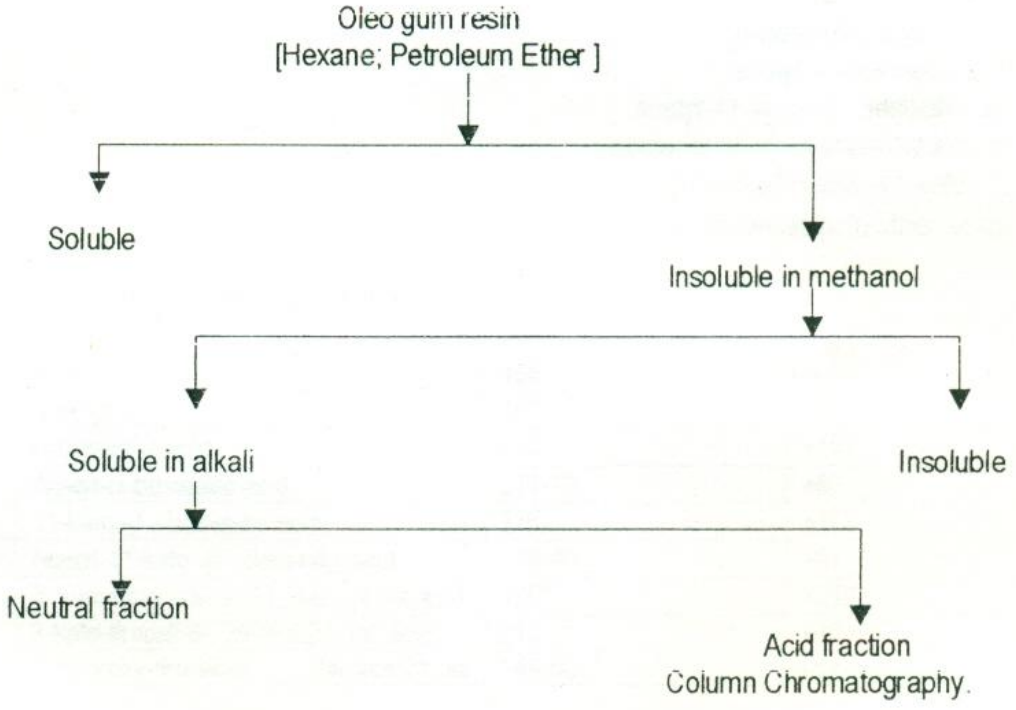
Method I

Gum resin of B. serrata which is crushed to small lumps or coarse powder is first extracted with n-hexane or petroleum ether and then with methane. The methanolic extract is separated into natural and acidic fractions by treatment with alkali. For the separation of individual acids may be achived by coluomn chromatography over silica gel. (flow chart I)

Method II

Triterpenoic acids in the form of acetates may be isolated by the modified methods of winterstein et al 31. The crushed lumps or powdered gum exudates are extracted with solvent or medium polarity such as diethyl ether, acetone, or ethyl acetate etc., and the total acids are precipitated as their barium salts. The barium salt on treatment with acetic-anhydride form a mixture of triterpenoic acid acetate. Repeated crystallization from the mixture results in the separation of acetyl - β- boswellic acid and acetyl-11-keto-β - boswellic acid in pure form. These mixtures may also be separated by coloumn chromatography over silical gel. Pure β- boswellic acid and 11-keto-β-boswellic acid are obtained by acid or alkaline hydrolysis of corresponding acetates (flow sheet 2)

Flow sheet .I



Flow Sheet II

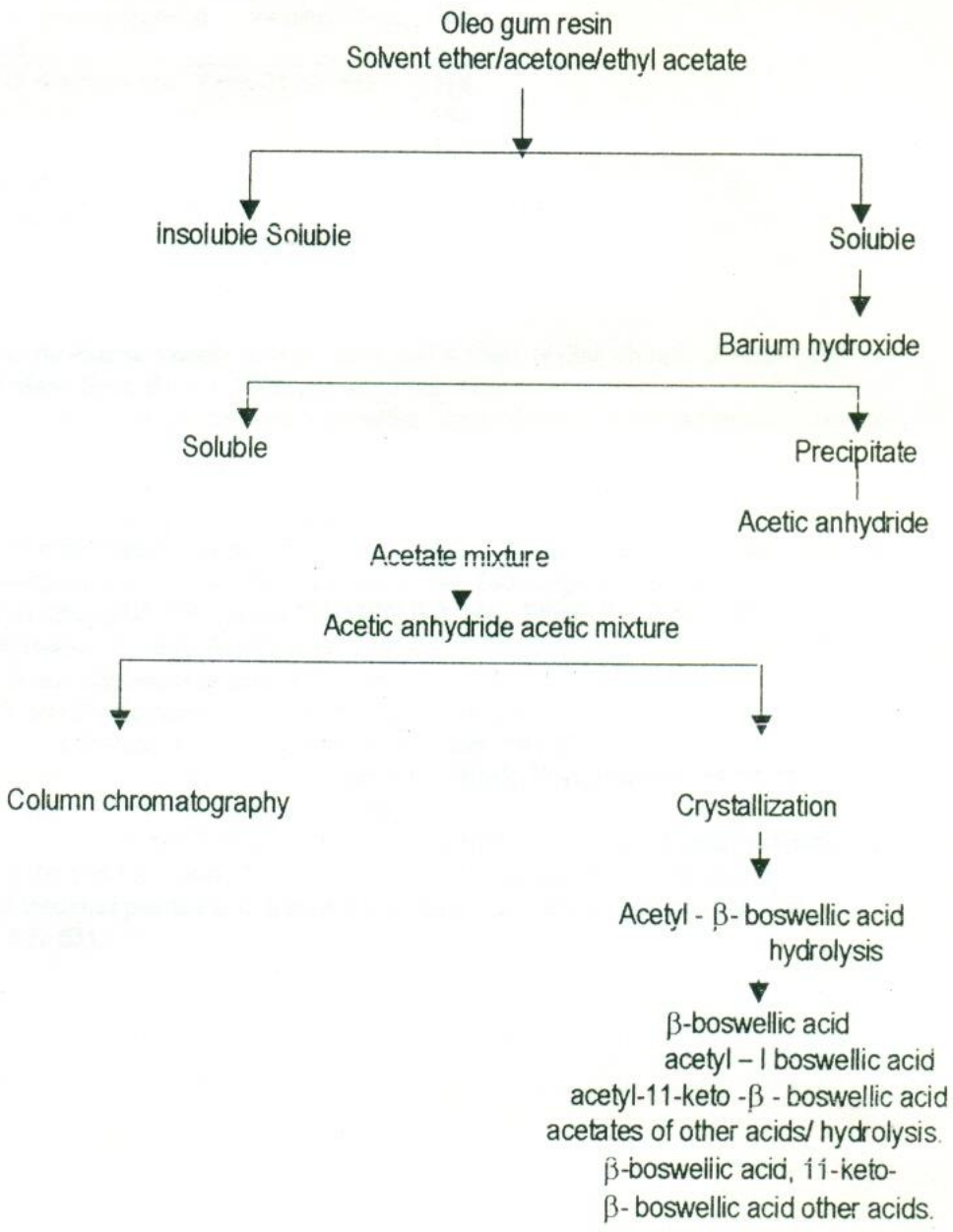


Table no. 20. Values of chemicals isolated.

S.No.	Compound	M.P (C°)	[α] D
1.	Amyrin	186	+91
2.	α-amyrin	197	+79
3.	α-boswellic acid	232	+107
4.	Acetyl-α-boswellic acid	271-73	+63
5.	11-keto -β - boswellic acid	270	+128
6.	Acetyl-11-keto -β - boswellic acid	238-46	+87
7..	3 α-acetoxy urs- 9, 11, dien-24-oic acid	175*	+338
8.	3-keto-tirucall-8-, 29-dien21- ioc acid	212	+28
9	3'-hydroxy-tirucall-8, 24-dien-21-oic	149-50	- 11

	acid		
10.	3' acetoxy- tirucall-8, 24-dien-21-oic acid		
11.	3-α-hydroxy-tirucall-8, 24-dien-21-oic acid	186	-2
12	2'-3'-dihydroxy urs-12 ene-24-oic acid	174	-
13.	Urs-12-ene-3'-24 diol	184	+69
14.	Urs-12-ene3', 24 diol	180	
15.	Serratol	-	-93

* Methyl ester

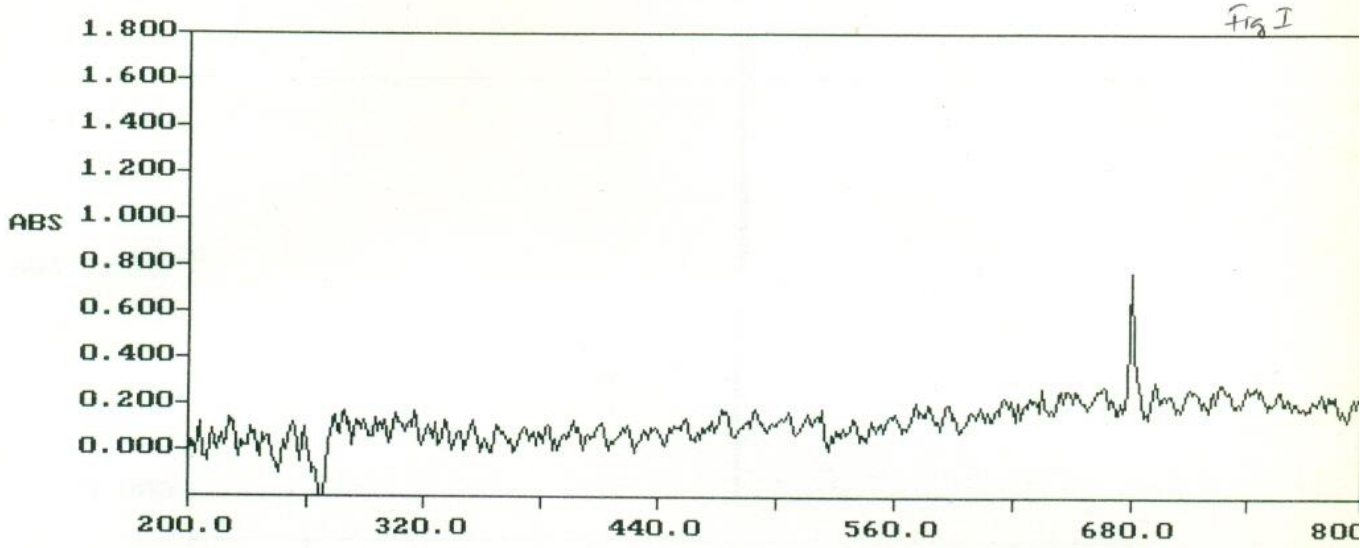
References:

1. The Ayurvedic Pharmacopoeia of India, vol-1, part-II, Dept. of ISM, Ministry of Health and Family Welfare, Govt. of India, 1999, appedix-2, pp. 183.
2. Anonymous., Manual for Quality control of Herbal Drugs, National Botanical Research Institute, Lucknow-1, 2001. Chapters 2, 3.
3. C.K. Kokate, A.P Purohit, S.B. gokhale., Pharmacognosy.,Nirali Prakashan2002.
4. Pearson, R.S and singh, d., (1918). Indian forest Records, 6.321; Simdon, J.L. and owen, L.N., Vol 2 (1949). The terpenes University press , Cambridge, p.10; Guenther E.S., (1943). Am perfumer, 45, 41). Bhargava, p.p., (1963). Per.f.Ess. oil Rec.,54, 740; Girgune, J.B, and Garg, B.D., (1979). I. Science. Res (Bhopal). I, 119; Dennis T.J (1980) Bull. Med. Ethno. Bot. Res., 1, 353.
5. Tschirch. A; Halbey, O, 1898, Ach Pharma, 236, 487
6-7. Pardhy, R.S, and Bhattacharya, S.C., (1978) Ind. J. Chem, 16(b), 191. 26.
Pardhy R.S. and Bhattacharya S,C., (1978), Ind J. Chem. 16 (B) 174
Pardhy R.S. and Bhattacharya S,C., (1978), Ind J. Chem. 16 (B) 176.
8. Mahajan B., Sethi, V.K. Taneja, S.C and Dhar, K.L., (1995), Phytochemistry 39 (2), 453
9. Malenclkar, M.A., (1925) J. Ind, Inst Sci., 8, 240.
10. Studies towards the development of a New Anti-inflammatory Drug from Boswellia serrata Gum resin. S.Ctaneja &K.L Dhar., R.R.L Jammu-180001. Supplement to cultivation & Utilizationof Medicinal plants Ed. S. handa & M.A. kaul. R.RL, CSIR, Jammu-Tawi 1996, Niscom. P. 527-531.

SYSTRONICS 119

DATE: 27-6-2002 TIME: 13:01

Fig I



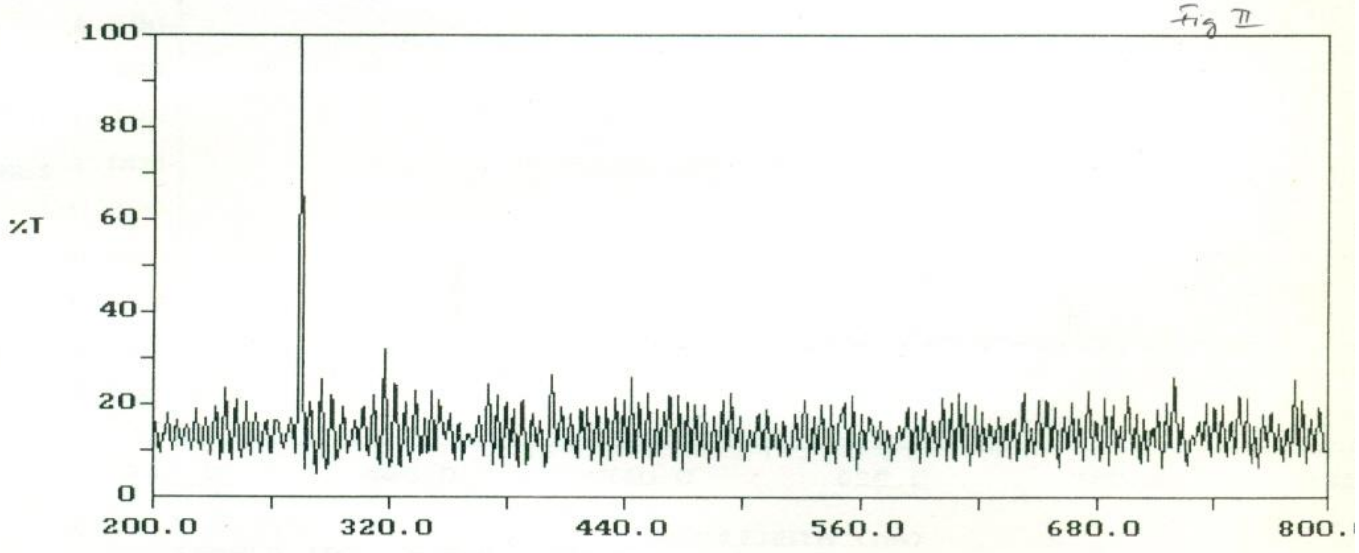
SAMPLE ID: kundur2

PEAK PICK		POINT PICK	
680.0	0.769	200.0	-0.038
692.0	0.292		
634.4	0.263		
710.0	0.263		
722.0	0.257		

SYSTRONICS 119

DATE: 27-6-2002 TIME: 14:07

Fig II

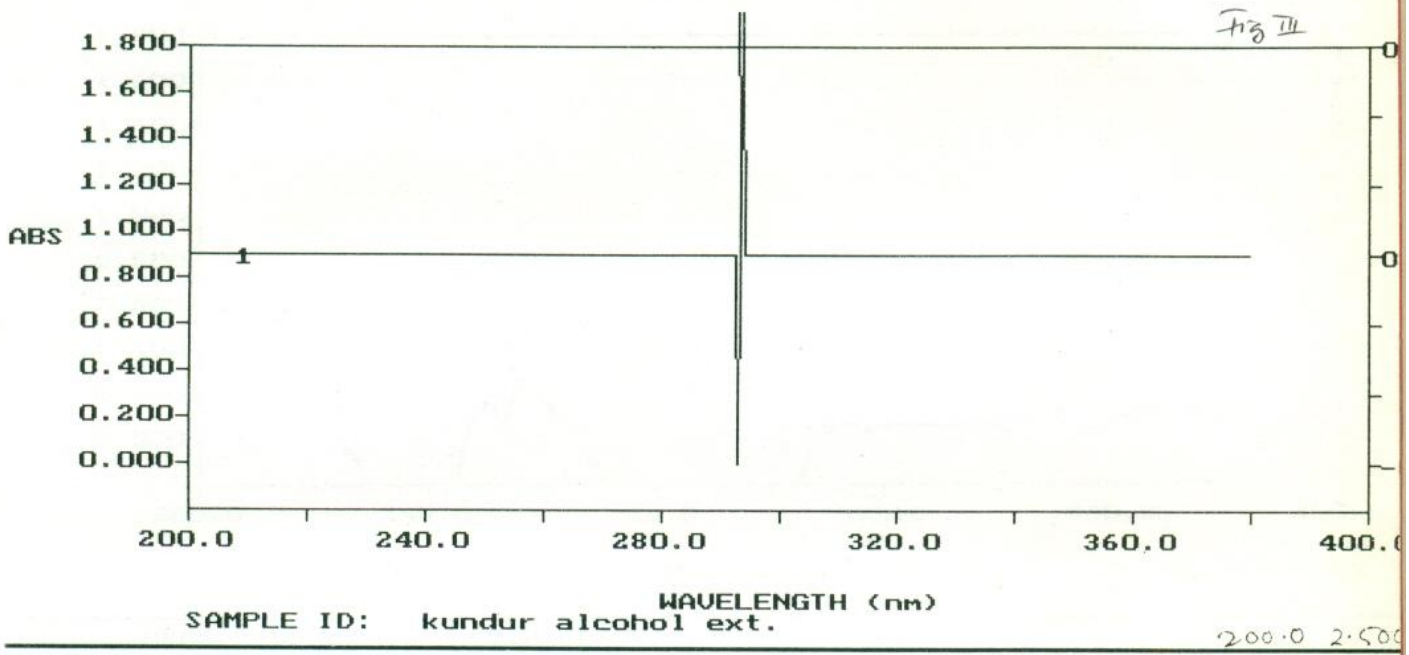


SAMPLE ID: bark

200.0 175

SYSTRONICS 119

DATE: 29-6-2002 TIME: 12:08



SAMPLE ID: kundur alcohol ext.

200.0 2.500

Reading	Wavelength	Ord.1	Conc.1	Ord.2	Conc.2
1.	200.0	0.900	36.00%		

SYSTRONICS 119

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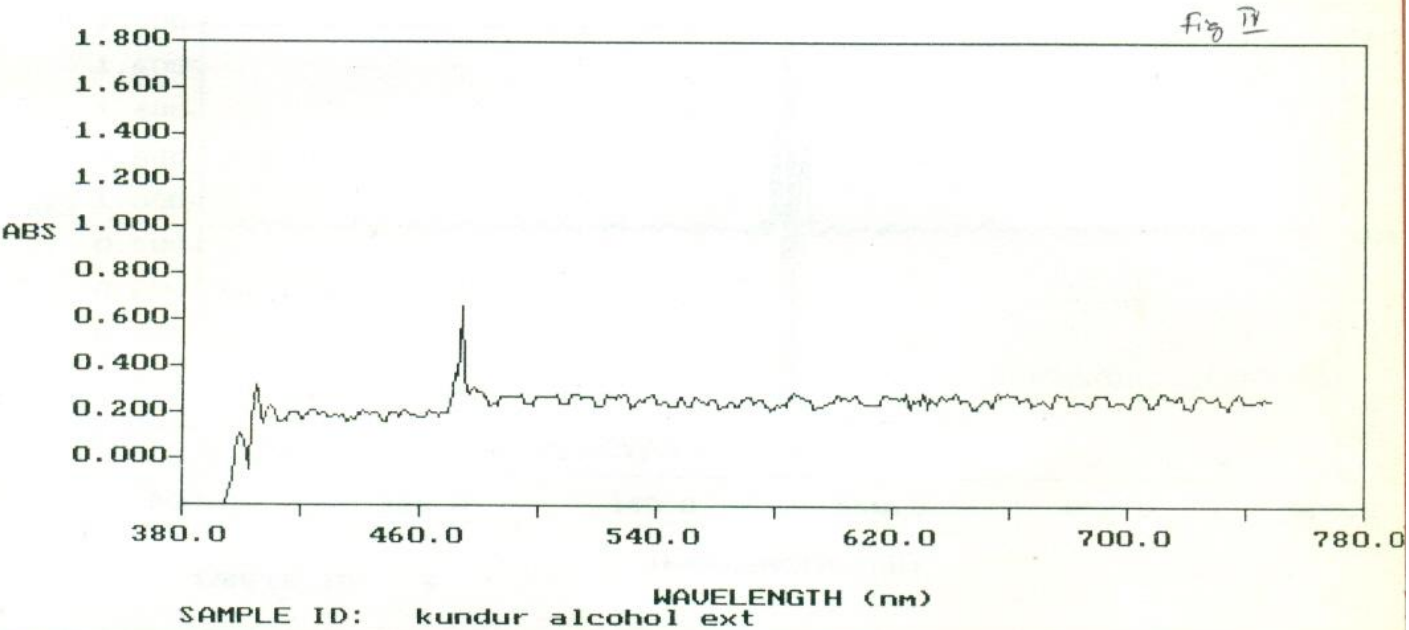


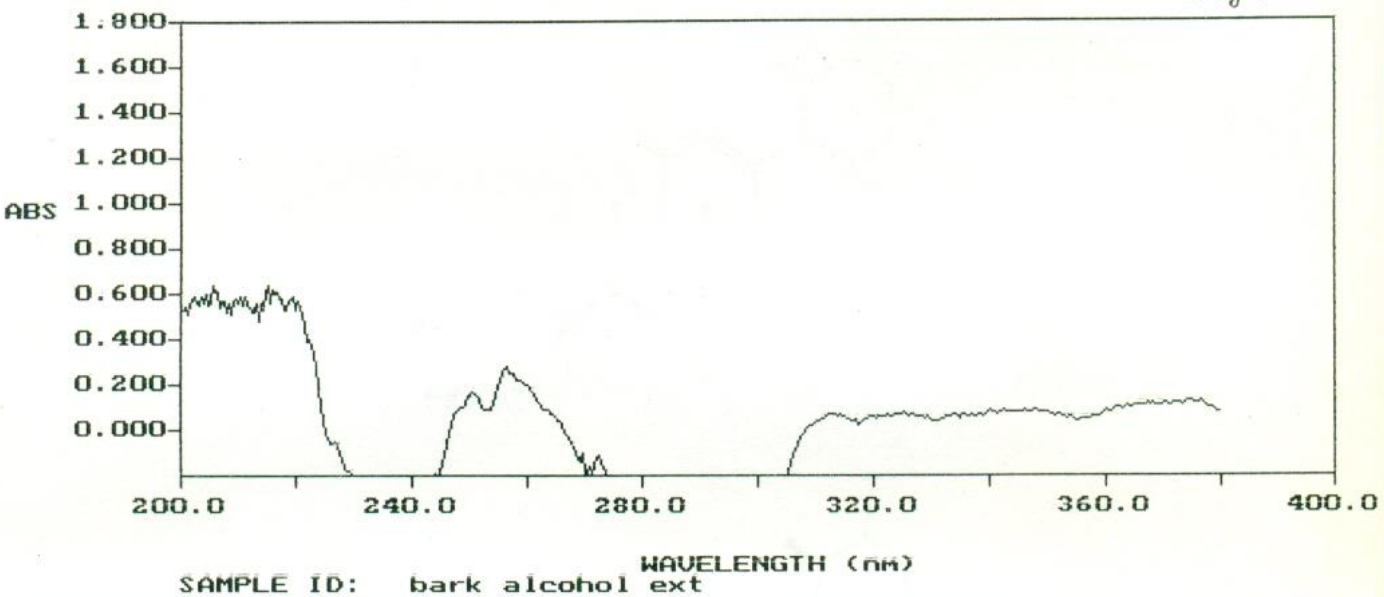
SAMPLE ID: kundur alcohol ext

PEAK PICK		POINT PICK	
474.4	0.653	380.0	-0.567
472.0	0.369		
404.8	0.317		
399.2	0.101		
384.0	-0.544		

SYSTRONICS 119

DATE: 29-6-2002 TIME: 15:02

Fig I



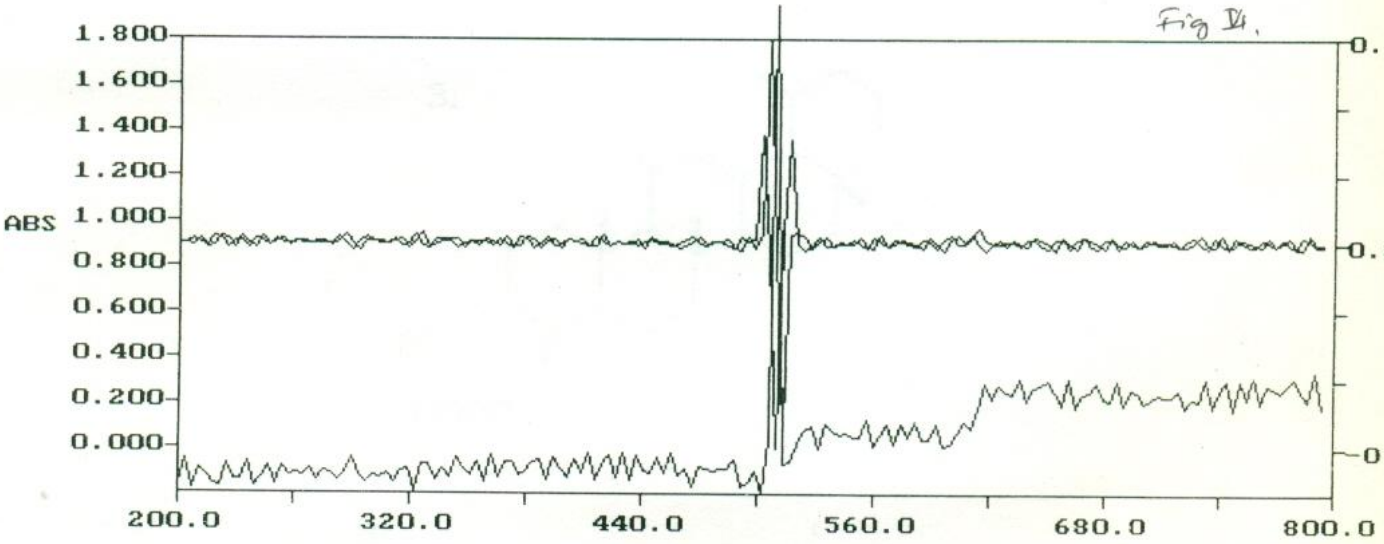
SAMPLE ID: bark alcohol ext

PEAK PICK		POINT PICK	
205.6	0.635	200.0	0.504
215.2	0.632		
256.4	0.270		
248.4	0.097		
306.8	0.020		

SYSTRONICS 119

DATE: 15-6-2002 TIME: 18:03

Fig II.



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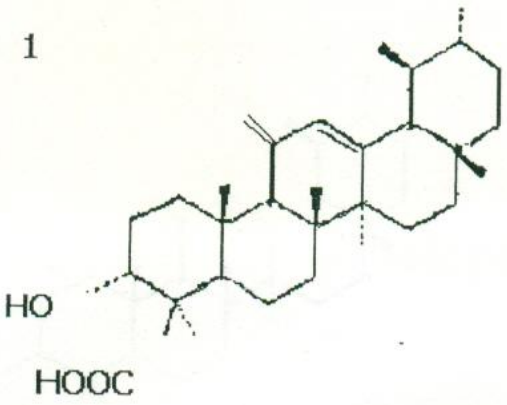

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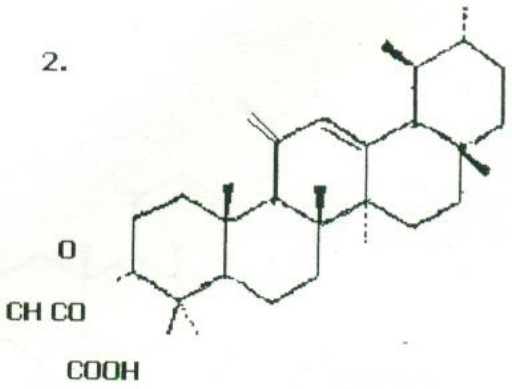

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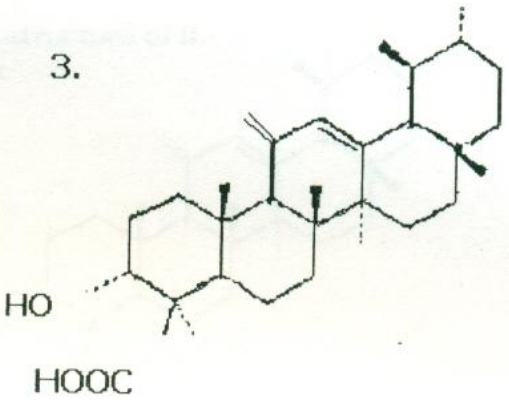

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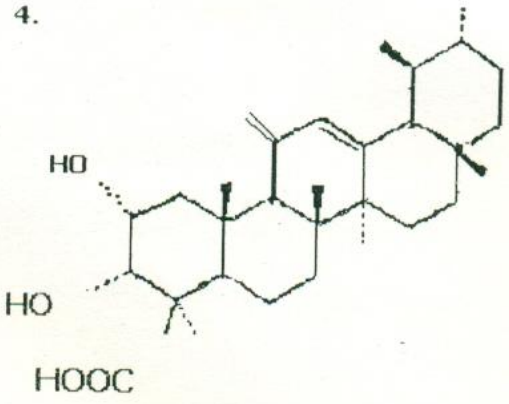

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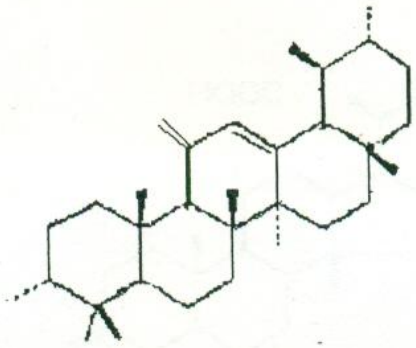

CH OH

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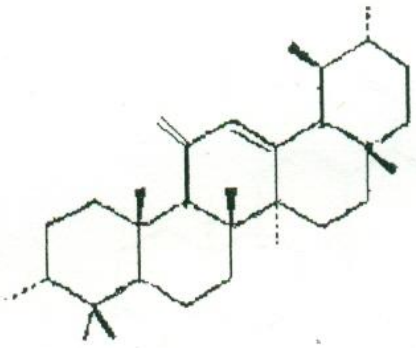
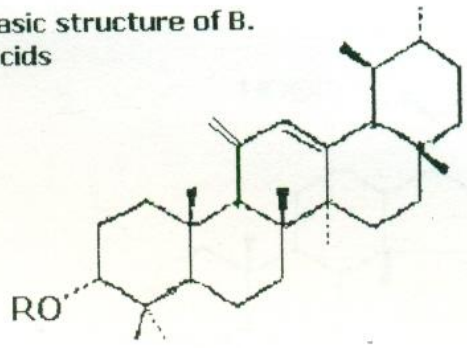


HO

basic structure of B. Acids

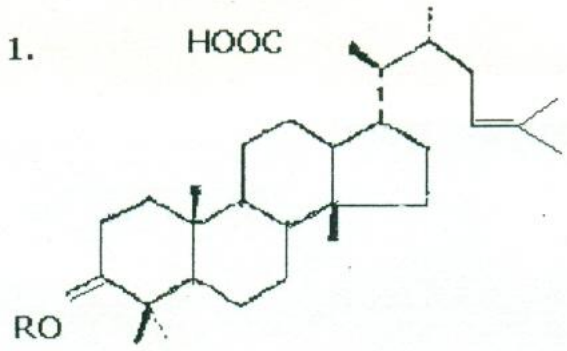


RO

HOOC

Tetracyclic Triterpinoids

1.



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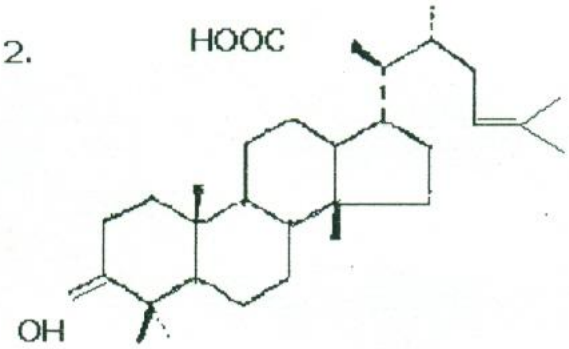

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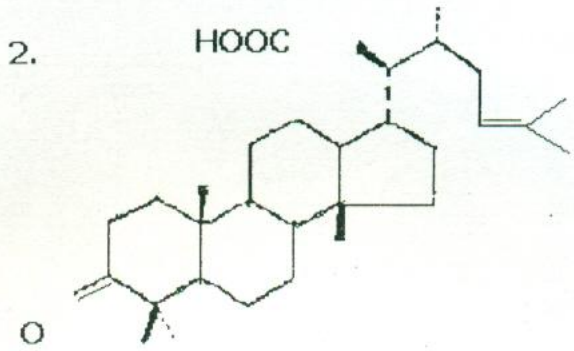
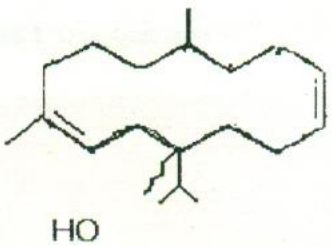


And
Serratol



TLC [P.Ether extract on 60/40 solvent: Petroleum ether]

Discussion & Summery

10. Discussion and Summery

Standardization

With herbal remedies accurate methods of assay are often lacking, and in instances where the active constituents are unknown there is no means of assessing therapeutic potency except be the use of a biological assay, should this be feasible. On the herbalist's contention that it is the effect of the complete extract of the plant that is most desirable, then perhaps the biological assay is the one which will reflect the true activity of the drug most clearly.

Fortunately it is possible to subject all crude botanicals to a pharmacological examination involving authentication, freedom from impurities, simple extractive tests and general quality control before they are extracted. This done, then with in the limits of variation caused be chemical races, ontogeny and geographical source all preparations made from different consignments be sound manufacturing methods should be generally acceptable and of uniform quality. *

**Trease & Evan's Pharmacognosy. WB Saunders Company Ltd. 14th ed. 1989, 476.*

The very aim and spirit of Dravyaguna vygyan is to provide the exact details of any substance which are used for human consumption be it for food, shelter, clothing, medicine and what so ever. The very importance of Ayurveda is two fold i.e., the alleviation of disease in the suffering and protection of health in a healthy individual -स्वस्थस्य स्वास्थ्य रक्षणम् । आतुरस्य विकार प्रशमनं च । To attain success in any treatment quality medicines are a must.

The aspect of providing with a suitable medicine which is having enough potency(virya) to deliver the desired therapeutic effects needs meticulous understanding of the qualities (gunas) of the material, and its Saviryata (proper shelf life) the crux of Ayurvedic specialization Dravyaguna lies with developing proper parameters, which ensure the quality of the drug as prescribed in the Texts which will ensure the therapeutic benefits attached to it.

The contemporary Dravyaguna specialists work becomes further complicated as there is serious scarcity of Raw materials due to de-forestation , and phenomenal demand for herbs in the domestic as well as in global market. This has only lead to unscrupulous practices leading to the development of standards of almost all herbal raw materials.

Drug standardization is multi faceted discipline and Ayurveda vaidyas has to work hand in hand with other specialists involved in the field such as Taxonomist, Botanist, Pharmacognosy expert, Phyto-chemist, ethno-botanist, people from tribes, and other sections of people.

Ayurveda has rich documented tradition (codified Texts viz., Samhitas, Nighantu, Shabda kosha etc.) . There fore it becomes mandatory for any drug which finds its place in ayurvedic texts need through documentation i.e., collection of data from as many texts as possible. The second step is to analyze the nomenclature, morphological characters described in them, the therapeutic and other cultural uses attributed them so that modern parallels can be drawn and the correct identification can be achieved .

The theory of pharmacolinguistics is elaborated in the first part of conceptual study and the results of its application related to the drug shallaki are discussed in the first part of materials and Methods.

The results of the study

a. there are around 25 synonyms for Shallaki plant as whole are described – thy are: *Shallaki, sallaki, surabhi, hladini/hradini, gajapriya, maherana, rasa, mocha/mochi, gandhapatra, suradru, hrada/hlada, susrava, suvaha, vanya, maheranya/maherana/maharuha, aswamutrika, gandhakari, vallaki, vanaparnika, gajabhakshya, satikta, atirasa* etc.

b. There are 22 synonyms given to *shallaki niryasa – khapura, kunduru, kundu/kunda, mkundah, bhishana, bali, naga, shrivallabha, svaksha, meduka, kunduruki, shikhari, kundragopura, sukundra, teekshagandha/teekshna, palinda, kunduruka, sourastra, gopuraka, bahugandha, mechaka* etc.

List of Vernacular names (local names)

Different regional names (Indian) of Shallaki are given below :-

Bengali: *Kundur , Luban, Salai,* **Hindi** : *Kundur, Luban, Salai, Lohban* , **Gujrati**: *Dhup, Gugali, Mukul, Salai,* **Kannada**: *Chitta, Gugguludhupa, Madimara,* **Malayalam** : *Gugguludhupam, Sambrani,* **Tamil** : *Kundrikam, Konkani: Visheshdhup,* **Telugu**: *Phirangisambrani, Parangisambrani* **Marathi**: *Salai, , Salphullic, PahadiDhup, Vishesdhup,* **Oriya** : *Loban, Gonda : Bor-salla, Santal: Salga,* **Deccan** : *Kundur,* **Bombay**: *Gandh-biroza, salai*

Foreign Languages:

Eng: Indian Olibanum, Indian Frankincense, **French**: Boswellie-dentelee,

German: Indischeweihrauch-baum **Arabic** : Olibanum, Kundur, Luban

Taxonomic status of shallaki

Kingdom	- Plant
Division	- Spermatophyta
Sub-division	- Dicotyledane
Sub class	- Polypetalae
Series	- Thalamyflorae*
Order (21)	- Geraminales
Family	- Burseraceae
Genus	- Boswellia
Species	- serrata.Roxb.*

Etymology of the Scientific terminology:

Boswellia = After Dr. Boswel, Edinburgh, serrata= saw toothed;

B.thurifera= yielding frankinscence;

B. glabra= hairless

Therapeutic considerations after referring the Texts are:

Rasa: *Madhura, Katu, Kashaya*

Guna: *Sheeta, Teekshna, Ushna, Snidga.*

Virya: *Sheeta*

Vipaka: *Katu*

Samanya karma:

a. **Dosha**-*Kaphahara , Pittahara, Vatahara*

b. **Dathu**-*Medohara, Raktaprasadaka, Pustikrit, Twachya*

c. **Mala**: *Purishavirajaneeya Swedapaha*

d. **Others**: *Arocha, nashak, Jwarahara, Graha peedahara, Alakshmihara, Sthambhi/ Grahi Varnya, Vishanashaka, Yonidoshahara.*

e. **Vyadhi**- *Vrana, Arshas (fruit), Kusta, Pakwatisara, Raktitisara, Rakta pitta, Pradara, Yuka, Mukha roga, Udaramaya, Kandu, Pidaka, Kota, Bhagna, Upadamsha, Swasha.*

Importance of the drug:

A. Unanimous acceptance (non-controversial drug)

Use of Shallaki and its niryasa (kundur) are as old as the samhitas. They find place in almost all Ayurvedic Texts and the drug which is unanimously accepted as Shallaki and further botanically identified so is Boswellia serrata Roxb. Ex. Colebr.

B. Easy to identify

A medium to large sized deciduous balasmiferous tree, up to 18 m in height and 2.4 m in girth (normally 1.5 m) commonly found in the dry forests from Punjab to west Bengal and peninsular India. Bark greenish grey , yellow reddish grey, fairly thick, smooth, exfoliating in thin papery flakes, resinous inside, leaves imparipinnate, 30-45 cm long; leaf lets 2-5- 6.3 cm *1.2-3.0 cm o9vate of ovate lanceolate, variable flowers small, shite,in axillary racemouse of panicles; droops 12mm long, trigonous, splitting along 3 valves, pyrenes compressed, hard, winged.

C. Wide availability

The tree is common at the foot of western Himalayas, in Rajasthan, Gujrat, Maharastra, Madhyapracesh, bihar, Orisa, Andhrapradesh and further south peninsula. The tree forms almost pure forests yielding and abundant supply timber. Large forests of this tree occur in the Khandesh and Nagpur-wardha divisions in Mahatstra, and Khadwa-Nimar division in Madhyaprdesh, Adilabad in Andhrapradesh.

D. Jangala pradesha drug (tough species – rate of survival is highest)

This tree prefers dry, hot exposure of rocky hills, especially the volcanic traps. On poor and shallow soil or where the ground is ferruginous, it is found pure, or nearly so. In North India, it is found on the southern slopes of the Siwalik hills, flanking the Himalaya on the dry sandstone ridges associated with *Acasia catechu, Anogeissus latifolia, Buchanania lanzan Spreng, Lannea caromandelica (Houtt) marill, Nyctanthus arbor-tristis Linn, Ziziphus xylopyra* etc.

In Rajasthan, it occupies the drier ridges of hills on metamorphic rocks, chiefly gneiss and schist, and occurs either pure of mixed with *Lennea coromandelica, Stercuiia urens Roxb.* , etc, In central and peninsular India it occupies the hotter slopes and ridges, usually on trap and sometimes on gneiss and schist associated with *Acasia catechu, Anogeissus latifolia, Lannea coromandelica,*

S. *urens*, and sometimes *Hardwickia binata Roxb*, and *Tectona grandis* Linn. F. the tree is able to thrive and reach fair dimensions even where other species remain stunted. In its natural habitat, the absolute maximum shade temp varies from 38-49° C and the absolute minimum from –1to 7° C and the normal rainfall ranges from 50-125 Cm

Guggul vis-à-vis Salai guggul

Guggulu forms the bulk of Ayurvedic formulations which are meant for treating various Vata and Kapha Vikaras. Interestingly in India we come across and use different types of Oleo-gum-resins such as Kan guggul, Salai guggul, Hira bol, Sarjars etc.

Amongst these two most important products come from the Burseraseae family i.e. Commiphora mukul and Boswellia serrata which yield Kan Guggulu and Salai guggul respectively. Amongst these two the former is used widely and also studied thoroughly well. It finds its uses as an effective anti-inflammatory agent, as well as an anti-cholesterolaemic drug. Unfortunately the Commiphora species is under threat due to excessive tapping and its use as fuel by people especially in Rajasthan region. This situation has warranted to search for a viable alternative to Guggulu and Salai guggul finds a quick place with similar effectiveness as an anti inflammatory agent and equally good lipid lowering drug.

Shallaki Guggulu –Most happening Ayurvedic Drug

The unique quality of Leucotriene [inflammatory mediators of lipoxyginase cycle] inhibiting activity[6] of Shallaki [Boswellic acids] has made it a special drug. The clinical studies also have shown considerable anti arthritic activity which has increased its demand tremendously. Many Pharmaceutical companies are marketing it either as a single herb or as a compound preparation.

The drug also has a prospect of its effective use in Asthma in which other NSAIDs generally precipitate the condition. It is well known fact that Leukotrienes play a major role in development and complications of asthma. The boswellic acid s being potent leuko-triene inhibitors may play a vital role as asthma preventing and managing agents which can become another thrust are for exploring this drug in this aspect.

Importance of *Twak* and *Twak churna*.

References from *Susrutha Samhita* and *Charaka Samhita* imply that apart from the use of *niryasa* [gum resin] , use of *twak* and *twak churna* [bark &bark powder] is also recommended for treating conditions like- upadamsha vrana, swasa vyadhi which speak about the efficacy and utility of twak churna. In spite of the availability of this drug usage of twak churna is seldom put to use by any vaidya With due consideration for usage of *Shallaki twak churna* and the possible scope its utility as substitute for *niryasa*, it has been strongly felt that developing standards for *twak* and *twak churna* are essential, which will beneficial for the future clinical work .

Quality control

a. Market nomenclature:

Frank incense and Indian frank incense find very important place in Unani medicine. They call it kundur – luban and is used generally to treat the Urinary tract disorders. We find mention of several kinds of Olibanum such as :

1. Deep yellow tears, called Kundur Zakar, or Male frankincense
2. pale tears called kundur Unsa , female frank incense
3. Kundur madahraj, artificial tears made by shaking the moist exudation in a basket
4. Kishar Kundur or Kashfa the bark or scurf of the tree is coated with the exudation (Dhup of Bombay market)
5. Dukak Kundur, or dust of Olibanum. The first kind is most esteemed.

b. Grading

Generally four grades of Kundur [gum rosin]

1. Superfine grade : is translucent, very light yellow in color, free from bark and other bark and other impurities;
2. Quality I: is brownish, less translucent and free from bark and other impurities.
3. Quality II: is brownish, semi translucent and may have some impurities;
4. Quality III: is dark brown, opaque, and with impurities. The percentage recovery of these grades varies from 10-13, 18 to 20, 20-25 and 25-30, respectively. The remaining may be left as a waste in the form of bark and impurities is also sometimes sold.

Shallaki and other drugs which are sold in the same name

An interesting observation is made during the study of the marketing trends of this drug. In Hyderabad market the venders are selling colophony like dry resinous matter (they also call it Papdi lakh- and it is told that it is generally procured by painters) in the name of Shallaki and they are providing the kundur in the name of LOBAN.

In jaipur market also the venders are not at all conversed with the term Shallaki and to procure Kundur – the shallaki niryas one has to ask for "kundur" or" dhup gond." In the Mumbai market it is termed as gandh biroj and generally the terms loban relate it to Frank incense which is imported. No market sells the bark and it has to be collected from source plant only.

To verify the differences between the sample sold in the name of shallaki from Hyderabad market that sample is also included in the study along with other oleo- gum- resin samples. The study as elaborated further has clearly revealed that the drug which is termed spuriously as Shallaki is not at all related to the source plant Boswellia serrata.

It is the old remains of turpentine distillation (colophony) and discarded material which do not have any therapeutic effect, and unfortunately is sold at a higher price than that of original Kundur (a difference of at least Rs. 200).

c. Microscopic characters:

Standard pharmacological procedures have been followed as described in section Materials and methods- part II. The results of the study are as follows:

a. Bark

The sample shows Bark cells[bast tissue, companion cells, , crystals, compactly packed brownish colored pigment cell clusters, paranchyma cells Lignified elements, and sieve tubes are seen.

b. Microscopic Characters of oleo-gum-resins powder:

Gum: alcohol dissolves resin, leaving angular non crystalline particles of gum and fragments of bark. Powder when treated with %% NaOH and 5% FeCl3 no change in color occur either it gives any stain when pressed between filter paper.

Gum shows irregular [angular] shaped transparent gum, opaque ,blackish[resinous material], plant residue is seen.

Microscopic characters (Sections of Shallaki)

The transverse and longitudinal sections of the young stem as well as the old stem are made from the fresh cuttings and the dried specimens also. Details of the study are mentioned in Materials and methods part II. The interesting aspect of the microscopic study is the shizolysigenous ducts which are the biological factories of Shallaki niryas. A closure look at these ducts reveal many interesting details which are as follows:

Ultra-microscopic details or Boswellia serrata Roxb. Stem and the Oleo-gum-resin duct (Shozolysigenous ducts.)

Oleo-gum-resin ducts are present in primary and Secondary phloem. The Duct lumen forms and enlarged apoplastic space surrounded by epithelial cells. The epithelial cells are rich in dictyosomes, lipid bodies, mitochondria with dilated cristae, multi vesicular bodies, osmiophilic materials, plastids and vesicles. Plastids have poorly developed internal membranes. Dictyosomes and plastids are possible sites of resin synthesis. The gum component of the exude is formed in dictysomes and from the outer layers of the inner tangential wall (wall facing the duct lumen). This wall is replenished from inside by the activity of dictyosomes.

The secretary materials are transported to the apoplast by granulocrine and eccrine secretion. They migrate through the loose micro-fibrils of the inner tangential wall into the duct lumen. Rarely, epithelial cells of young ducts have rudimentary plasmodesmata on the inner tangential wall, which may be channels for passage of secretary materials into the duct lumen.

d. Physical and chemical Assay

The study for Physical and chemical Assay, purity, strength have been performed according to standard pharmacognostic methods as described in Ayuvedic pharmacopoeia of India-part I. The conclusions of the study are as follows:

Note:

- *Hyderabad B- sample is very much different from the rest hence named so. It is included along with the Kundur – sallaki niryas for in all studies and finds place along with them in the tables. The values of it are not considered for the final value and are considered completely different from the rest.*
- *The final values are calculated as the averages of similar samples collected from various – a standard practice used as per Ayurvedic Pharmacopial Committee [A.P.I] recommendations.*

1. Foreign matter of the bark sampie . Table no 1

S.No.	Name of the place collected	Percentage(%)
1.	Thamsa forest, Nanded Dist.	2
2.	Keesara gutta,Hyderabad, A.P	1.89
3.	Khandwa, Madhya pradesh	3
		2.29%

Table no. 2 Foreign matter of The Oleo-gum-resin sample

S.No.	Name of the place collected	Percentage(%)
1.	Thamsa forest, Nanded Dist.	1
2.	Hyderabad,A. (Kundur)	5
3.	Hyderabad,B*	1
4.	Jaipur market	3
5.	Himalaya drug co.	Material in capsules.
	Final value	2.25%

The observations clearly show that the samples collected from resource plant and after processing has very less percent of foreign matter. Where as market samples has ver y high percentage of foreign matter.

Table No. 3 Moisture content of the Bark

S.No	Name of the place	percentage
1.	Thamsa, Nanded dist.	15
2.	Hyderabad	18.011
3.	Khandwa	15.697
	Final value	16.236%

Table no. 4. Moisture content of the Kundur.

S.No	Name of the place	percentage
1.	Thamsa, Nanded dist.	10.99
2.	Hyderabad A	10.94
3.	Hyd. B*	4.0521
4.	Jaipur	10.96
5.	Himalaya Drug Co.	10.87
		10.94%

3. Ash values

Total Ash of Bark Powder - Table no.5

S.no	Place of collection	Percentage of the Ash in %
1.	Nanded	8.46
2.	Hyderabad	7.9
3.	Khandwa, M.P	8.4
	Final value	8.25%

Table no.6 Total Ash of Kundur (Shallaki niryas)

S.No	Place of collection	Percentage of the Ash in %
1.	Nanded (collected from forest)	2.09
2.	Hyderabad [A]	2.0
3.	Hyderabad [B]*	0.11
4.	Jaipur Market	2.11
5.	Sample of Himalaya drug Co.	2.08
		2.07%

Table no.7 Acid insoluble Ash of Bark Powder

S.No	Place of collection	Percentage of the Ash in %
1.	Nanded	2.5
2.	Hyderabad	2.5
3.	Khandwa, M.P	2.6
	Final value	2.5%

Table no.8 Acid insoluble Ash of Kundur (Shallaki niryas)

S.No	Place of collection	Percentage of the Ash in %
1.	Nanded (Collected from Forest)	1.38
2.	Hyderabad [A]	1.49
3.	Hyderabad [B]	2.56
4.	Jaipur Market	1.9
5.	Sample of Himalaya drug Co.	1.43
	Final Value	1.4

Table no. 9 Water insoluble Ash of Bark Powder

S.No	Place of collection	Percentage of the Ash in %
1.	Nanded	3.21
2.	Hyderabad	3.1
3.	Khandwa, M.P	3.8
	Final value	3.36%

Table no.10 Water insoluble Ash of Kundur (Shallaki niryas)

S.No	Place of collection	Percentage of the Ash in %
1.	Nanded	1.72
2.	Hyderabad [A]	1.7
3.	Hyderabad [B]	1.8
4.	Jaipur Market	1.72
5.	Sample of Himalaya drug Co.	1.71
	Final value	1.7125

Extractive values are performed using alcohol, water and petroleum ether as extracts. The final values are calculated approximating the values to 5g and 100ml ; the original values taken are 2.5 g and 50 g of solvent. Hence the values are multiplied by 40 to obtain final value.

Table 11- Alcohol soluble extractive of bark powder

S.No.	Source	W Multiplied by 40Value in %
1.	Nanded	18.492
2.	Hyderabad,	18.792
3.	Khandwa, M.P	18.248
	Final value	18.51%

Table no.12- Alcohol soluble extractive of gum-oleo- resin powder

S.No.	Source	W Multiplied by 40 Value in %
1.	Nanded	56.848
2.	Hyderabad A	58.008
3.	Hyderabad B*	64.056**
4.	Jalpur	58.008
5.	Himalaya D.Co.	58.016
	Final value	57.72

Table 13- Water soluble extractive of bark powder

S.No.	Source	W Multiplied by 40Value in %
1.	Nanded	17.58
2.	Hyd	17.52
3.	Khandwa	17.68
	Final value	17.594

Table 14- Water soluble extractive of oleo-gum-resin powder

S.No.	Source	W Multiplied by 40 Value in %
1.	Nanded	14.288
2.	Hyd A	14.3560
3.	Hyd B*	
4.	Jaipur	14.152
5.	Himalaya D.co.	13.736
	Final value	14.133

* not soluble in water.

Table 15- Ether soluble extractive of bark powder

S.No.	Source	W Multiplied by 40 Value in %
1.	Nanded	17.58
2.	Hyd	17.68
3.	Khandwa	18.128
	Final value	17.796

Table 16- ether soluble extractive of gum

S.No	Source	W Multiplied by 40 Value in %
1.	Nanded	14.286
2.	Hyd A	13.956
3.	Hyd B*	4.644
4.	Jaipur	14.2
5.	Himalaya D.Co	14.72
	Final value	14.2905%

* dissolved in very little quantities and remained almost unchanged after drying.

Table no. 17. Volatile oil content of the Bark powder

S.no.	Place of collection	% V/W
1.	Nanded dist.	2
2.	Hyd	3
3.	Khandwa	2
	Final value	2.33

Table no. 18. Volatile oil content of the Gum

S.no	Place of collection	% V/W
1.	Nanded	8
2.	Hyd A	9
3.	Hyd B*	2*
4.	Jaipur	10
5.	H. D.co.	8
	Final value	8.75%

Thin Layer chromatography

The method and the materials used for chromatography are discussed in the chapter materials and methods part III. The results of the test are as follows:

1. a. The **alcoholic extract of Kundur** (gum oleo resin of shallaki) has developed spots with Rf values 0.18,0.27,0.45, 0.85 with petroleum ether as solvent.
   b. With acetone: chloroform: water (70:30: 2) as solvent – 0.88;
   c, Chloroform as solvent –0.66; (total 6 spots)
2. **The Petroleum ether extract of the Kundur** (gum oleo resin of shallaki) with
   a. Petroleum ether as solvent developed – 0.64,0.70,0.76,0.83,0.91
   b. Chloroform as solvent developed – 0.75,0.92, 0.95 (8 spots)
3. The **alcohol extractive of the Bark** developed
   a. with Ether:Chloroform 30: 40 as solvent – 0.88,

4. Petroleum ether extractive and Ether as solvent – 0.61 (2 spots)
5. *the golden coloured sample (confirmed as the residue of colophohy) has not developed any type of chromatogram in an any extractive and type of solvent.

UV- VIS – spectrophotometry.

1. Spectra of Kundur (oleo –gum resin)Petroleum extract has shown peak absorbency at 680 nm.
2. Spectra of Percentage If transmittance of the Petroleum ether extract of Shallaki bark has shown a peak at 280nm.
3. Kundur – alcohol extract does not show any significant absorption in UV spectra). It shows peak pick at 474.4 nm in vis spectra.
4. Bark alcohol extract in UV- spectra shows peak absorption at 205.6 nm followed by other points at 215.2, 256.4, 248.4, 306.8 nm.
5. Bark alcohol extract shows peak pick at 500nm in the vis. Spectra.

Chemical composition

1. Terpenoids and higher triterpinoids:

The monoterpenes, identified in the oil include α– pinene, β- pinene ,α - thujene, δ- limonene, p-cymene, cadinene, geraniol, elemol, terpeneol, methyl chavicol and phallandrene , β- boswellic acid several other triterpinoids have also been isolated from the gum resin, these compounds include - amyrins, 11- keto - boswellic acid, 3'-bydroxy urs-9, 11-dien-24-oic acid . Tetracyclic triterpenoic acids, 3'-hydroxy-triurcall-8, 24-dien-21-oic acid, 3 keto-tirucall-8, 24-dien-21-oic acid ,
diterpene alcohol serratol, 21-3'-dihydroxy Urs-12-ene-24-oic acid and urs-12-ene-3'-24 diol.

2. Carbohydrates

Carbohydrates or sugars also constitute the major fraction (45-60%) of the gum resin. Preliminary examination indicated the presence of disacharides along with oligo and polysaccharides. Although not much work has been carried out in the gum resin come earlier work reported by Malandkar 30 et. al identified the acid hydrolyzed products as arabinose, xylose and galactose

Comparision between Kundur and the spurious sample [Hyd B- sample]

Table no.19.

S.no	Details	Kundur-shallaki niryas	Hyd-B sample
1.	Market Names	Kundur, Dhup, Luban, Gandh Biron, shallaki (companies)	Shallaki, Papdi-lakh, Colophony, Amber resin,pine- resin.
2.	Source	From Boswellia serrata Roxb.	Residue left after distillation ofturpentine from the crude- oleo-gum-resin obtained from various species of *Pinus* belong to Pinaceae family
3	Appearance	In small and large tears, dark brown to cream colour.	Angular, transluscent masses in various sizes, papery flakes, amber to golden yellow colour.

4.	Texture	Soft, gum like texture	Soft, brittle, flaky
5.	Odour	Characteristic turpentine like Agreeable on burning.	Faint and terbinthinate. Agreeable on burning.
6.	Solubility	Soluble in warm water with milk like appearance [characteristic feature] Soluble in alcohol and ether	not at all soluble in water [cold , warm , hot water- readily fusible] Soluble in alcohol; in ether the fresh samples are solubie; but the older the sample the difficult for it to get dissolved.
6.	Identity Purity , strength and assay.		
i.	Microscopic	Resinous martial, gum (transparent) , and bark tissue.	No plant material (bark cell etc.) are seen ; only translucent pieces in angular shapes are observed.
ii.	Foreign matter	2.25 % w/w	1%
iii.	Moisture value	10.94	4.0521
iv.	Total ash	2.07	0.11
v.	Acid insoluble ash	1.4	1.9
Vi	Water insol.ash	1.7125	1.8
**	Extractive values		
i.	Alcohol extractive	57.72	64.056**
ii.	Water extractive	14.153	Not soluble
iii.	Ether soluble (fixed oil content)	14.2905	4.644
***	Volatile oil content	8.75 % v/\w	2 % v/w
****	TLC	As per Table no. 19 – material and methods	Did not develop any chromatogram under similar conditions.
7.	Confirmatory tests		Produces bright red colour changing to violet in presence of acetic anhydride and one or two drops of sulphuric acid. Alcoholic solution is acidic to litmus.
8.	Marker compound	Boswellic acids	Abietic acid .
9.	Main uses	As medicine for treating Joint disorders, ulcers; preparation of incense and fixative in perfumes.	It is an ingredient, of certain plaster masses and collodions. Preparation of varnishes, insulating compounds, soaps, printing inks, paper sizing for floorings, soldering compounds, mastics and pressure sensitive adhesives.
10.	Remarks.	It is the original drug which deserves the names Kundur, and shallaki in all respects.	The name used for it as shallaki is only an misnomer and it has anything in common with the source plant B. serrata. Hence can be considered as a spurious drug in the name of shallaki.

Table no 20 . Comparison between Guggul and Salai guggul:

S.No	Details	Kundur-shallaki niryas	Guggul *
1.	Market Names	Indian- frank incense, Kundur, Dhup, Luban, Gandh Biron, shallaki (companies)	Scented Bdellium, Gum guggul, kan guggul.
2.	Source	From *Boswellia serrata Roxb.* Family: Burseraceae	*Commiphora weightii.* Family: Burseraceae
3	Appearance	In small and large tears, dark brown to cream colour.	Brown to pale yellow or dull green.
4.	Texture	Soft, gum like texture	Soft , gum like sticky.
5.	Odour	Characteristic turpentine like Agreeable on burning.	Agreeable, aromatic and balsamic.
6.	Taste	Bitter, acrid	Bitter.
7.	Solubility	Soluble in warm water with milk like appearance [characteristic feature] Soluble in alcohol and ether	Partly soluble in alcohol; when triturated with water, it forms white emulsion.
8.	Identity Purity , strength and assay.		
i.	Microscopic	Resinous martial, gum (transparent) , and bark tissue.	
ii.	Foreign matter	2.25 % w/w	4
iii	Moisture value	10.94	----
iv.	Total ash	2.07	5
v.	Acid insoluble ash	1.4	1
Vi	Water insol.ash	1.7125	5.62 *
**	Extractive values		
i.	Alcohol extractive	57.72	27
ii.	Water extractive	14.153	53
iii.	Ether soluble (fixed oil content)	14.2905	11.91 *
***	Volatile oil content	8.75 % v/w	1 % v/w
****	TLC	As per Table no 19 – material and methods	Details given in table no 21
9.	Marker compound	Boswellic acids	Guggulsterol 1
10.	Main uses	As medicine for treating Joint disorders, ulcers; preparation of incense and fixative in perfumes. More used in unani pharmacy for treating urinary tract diseases.	Main constituent of all guggul preparations used in ayurveda and Unani. Also promoted as cholesterol lowering drug
11.	Pharmacological considerations.		
	Rasa	Madhura Katu Tikta Kashaya	Tikta, katu, kashaya*
	Guna	Sheeta Teekshna Ushna Snidga	Laghu, ruksha, teekshna,* vishada,sukshma, sugandhi.*

	Veerya	Sheeta	Ushna*
	Vipaka	Katu	Katu*
	Dosha karma	Kaphahara ,Pittahara Vatahara	Kapha vata shamak*
	Dhatugata karma	Medohara, Raktaprasadaka	Medohara, *
	Malagata karma	Purishavirajaneeya Swedapaha	Malashodhan*
	Samanya karma	Pustikrit,,, Arochanashak, Twachyawarahara Graha, peedahara, Alakshmihara, Sthambhi/ Grahi, Varnya, Vishanashaka, Yonidosha hara.	Vedanasthapana, Kledanashana, amapachana. *
		Vrana, kusta, pakwatisara, rakatisara, raktapitta, pradara, yuka, mukha roga, udaramaya, kandu, pidaka, kota, bhana, upadamsha, swasha.	Amavata, sandhivat, apachi, gandamala, dusta vrana etc.*
	Others	Predominentlty Lipoxyginase cycle inhibitor Inhibits production of Lulotriens	Prediminently cyclo -oxygenase cycle inhibitor; inhibits prostaglandin production.
		Less ulcerogenic in g.i.t.	Has more tendency.
12.	Remarks.	It is the original drug which deserves the names Kundur, and shallaki in all respects.	Generally this drug is adulterated with resins of various commiphora sp. As c. toxhurghii,C. molmol and also with B. Serrata.
		They can not be substituted with each other. But when used in combination they are capable of arresting the complete inflammatory process which can provide superior results.	

*Drug standardization of single durgs –CCRUM; Ayurvedic pharmacopoeia of India part-I , I ed. Ministry of ISM, govt. of India, 1986; A.P Deshpande, R. R. javalgekar, S. Ranade-Dravyaguna vigyan , Anmol Prakashan,Pune -2

Table no.21. TLC values of guggul*

Extract	Solvent system	Spray/ treatment	No. of spots - details
Petr. Ether (60/80)	Petr ether, solu ether 4: 1	Iodine vapour	7- 0.16,0.20,0.39,0.44,0.74, 0.83,0.91.
Chloroform	Do	Do	2- 0.16,0.2

*Drug standardization of single drugs –CCRUM

Summery

Title of the work: Study on drug standardization of *Shallki Niryasa* [Gum resin] *and Twak Churna* [Bark powder]

Place of work: Department of Dravyaguna, Govt. Ayurved college& Hospital, Nanded, Drug testing Laboratory, Govt. Ayurved & Unani Rasashala, Nanded. Science College, Swami Ramanand Teerth Marathwada University, Nanded*.

Duration: Two years

Aim of the work:

- Botanical identification of the source plant *Shallaki.*
- Standardization of *Shallaki niryasa* [known as Kundaru] collected from source plant.
- Standardization of *Shallaki Twak* and *twak churna* collected from source plant.
- Survey of Market samples [of Shallaki niryasa] collected from different places for studying Purity and strength with respect to the source drug.
- Survey of samples from industry which manufacture medicines where *Shallaki niryasa* is used.
- Study of different collection and storage methods of *Shallaki nirysa* for better understanding and improvement.

Methodology:

Guidelines laid down by Ayurvedic pharmacopoeia and standard pharmacognosy techniques are followed for crude drug standardization, which are as follows:

1. Origin, common names, scientific nomenclature and family.
2. Geographical source (and history)
3. Cultivation, collection , preservation and storage
4. Macroscopic, microscopic and sensory [organoleptic] characters.
5. Chemical composition (whenever possible)
6. Identity, purity, strength and assay (quantitative data)
7. Determination of Quantitative data (Identity, purity, strength and assay)

i. Foreign matter determination
ii. Determination of total ash
iii. Determination of acid insoluble ash
iv. Determination of water in -soluble ash
v. Determination of alcohol soluble extractive
vi. Determination of water soluble extractive
vii. Determination of ether soluble extractive (fixed oil content)
ix. Determination of moisture content (loss on drying)
x. Determination of volatile oil
b. Special processes used in alkaloid assay
   i. Thin layer chromatography
   ii. ii. UV- VIS spectrophotometry.

8.Substitutes and adulterants

Plan of work.

a. resource plant identification

- For identification of source plant (which qualifies the description of *nighantu* and other Ayurvedic literature) standard botanical methods are followed[pharmacolinguistic standardization]

- Plants from different forest areas will be surveyed for studying the presence of any variants and other species.

b. Collection of samples

- 5- samples *of Kunduru* [*Shallaki niryas*] arecollected and evaluated for the parameters described above. [one from natural source[Thamsa forest, Nanded dist, M.S, two from markets[Hyderabad, Jaipur], one from Himalaya Drug company and another spuriously branded sample from Hyd. market – total 5]
- Three samples of Bark - fresh samples and dry samples are collected from different places[Nanded, Hyderabad, Khandwa, M.P], and evaluated for the parameters described above.

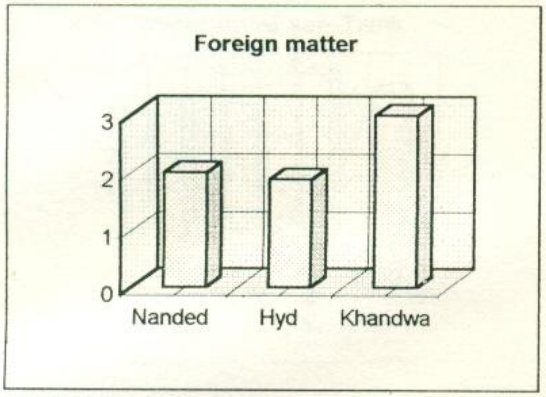
Results as per Ayurvedic Pharmacopoeia Of India (API) Format

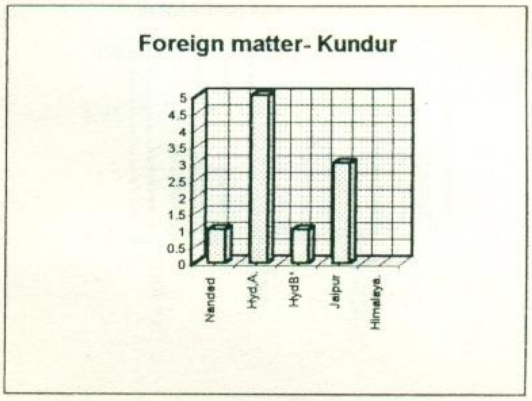
S.no	Details Title : Shallaki niryasa (Kundur) / Twak Chuma.	Results	
1.	Origin, common names, scientific nomenclature and family.	Details; Materials and methods Part- I	
2.	Geographical source (and history)	Do	
3.	Cultivation, collection , preservation and storage.	Do	
4.	Macroscopic, microscopic and sensory [organoleptic] characters.	Material and methods part- II	
5.	Chemical composition	Materials and methods part -III	
6.	Identity, purity, strength and assay	Gum	Bark
	i. Foreign matter determination	2.25% w/w	2.29
	ii. Determination of total ash	2.07	8.25
	iii. Determination of acid insoluble ash	1.4	2.5
	iv. Determination of water in -soluble ash	1.7125	3.36
	v. Determination of alcohol soluble extractive	57.72	18.51
	vi. Determination of water soluble extractive	14.152	17.594
	vii. Determination of ether soluble extractive	14.2905	17.796
	ix. Determination of moisture content (loss on drying)	10.94	16.263
	x. Determination of volatile oil	8.75 % v/w	2.33
	Thin layer chromatography	Material and methods part III	
	UV- VIS spectrophotometry.	Do	
8.	Classical attributes (asa panchak etc.,)	Materials and methods Part I	

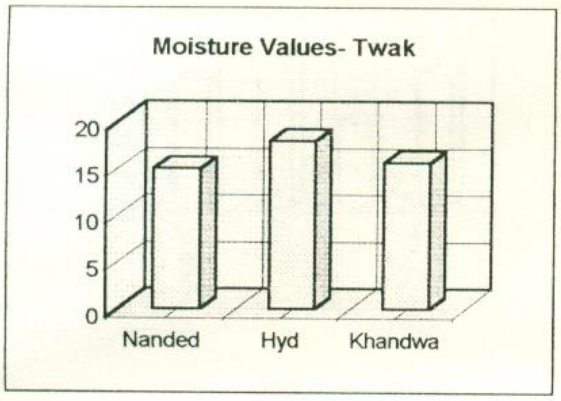
9.	Therapeutic uses	Do
10.	Important Formulations	Bala taila, Amritadya taila, Eladi taila .,
11.	Oral dosage	Gum powder: 1-3g; Gum dry extract : 250-750 mg Bark Powder: 2-4 g.
	Substitutes and adulterants	Adulterated with arabic gum and sold as a substitute of Guggul. A spurious drug in the name of shallaki is sold in Hyd. Market which is the residue of turpentine distillation.

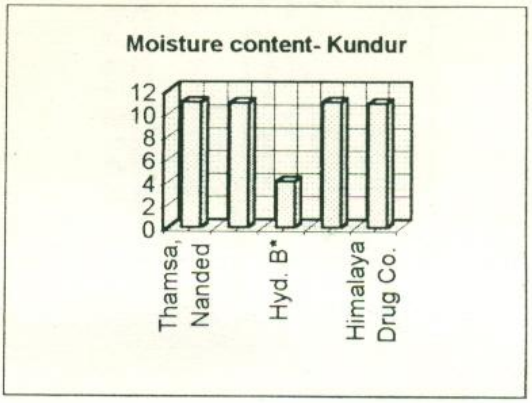
Note: The work is guided by Dr. Arvind Bhagwat and carried by MD scholar- Dr. Trigulla Saketaram , Department of Dravyaguna,G.A.C. Nanded. Expert guidance from Ayurvedic dravyaguna experts, reputed botanists, pharmacognosy experts and phytochemists, help from various reputed national libraries and internet is sought for completion of the work.

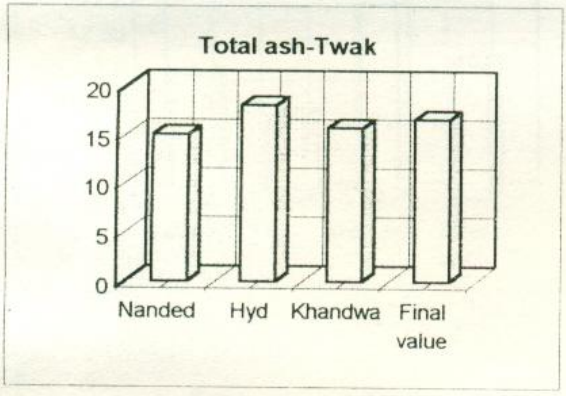
Graphical representation of the findings

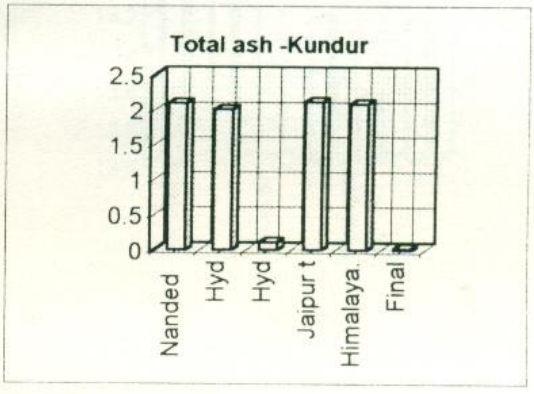


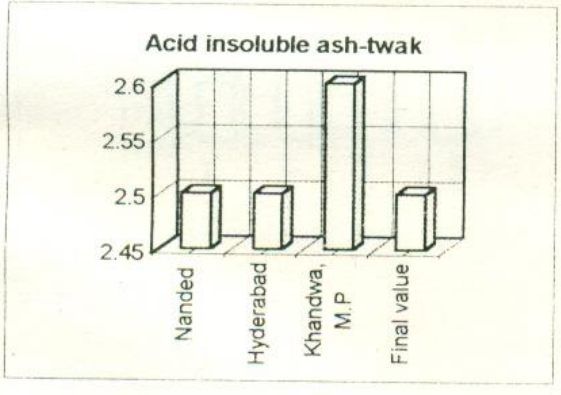


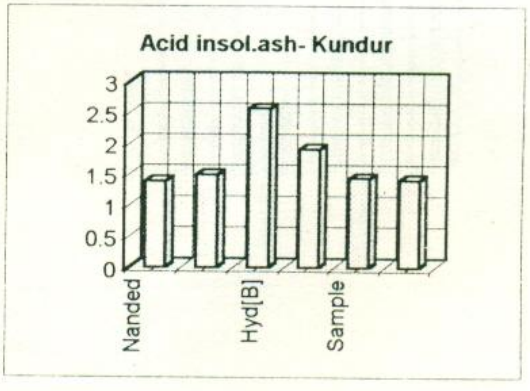












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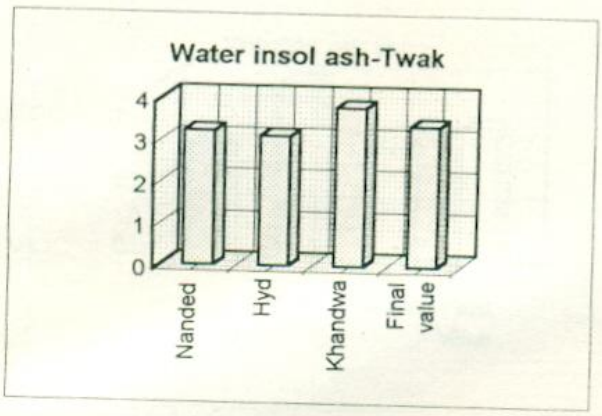
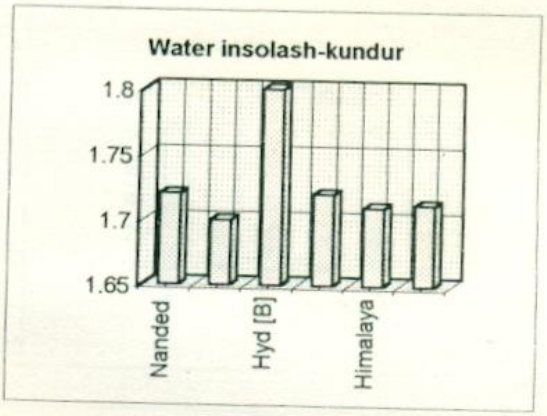
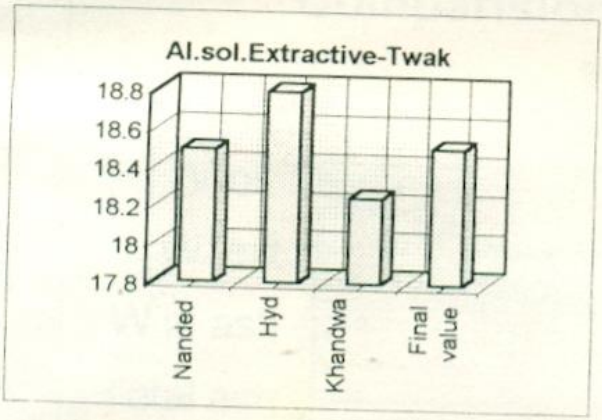
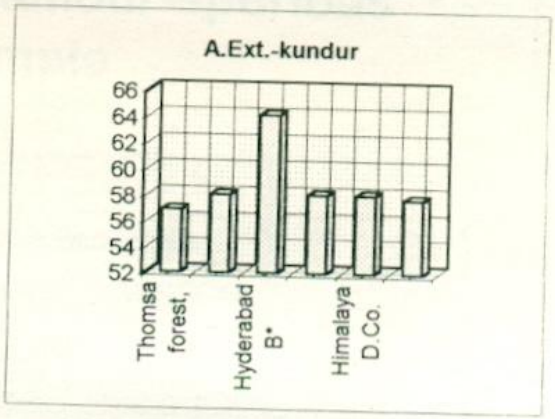
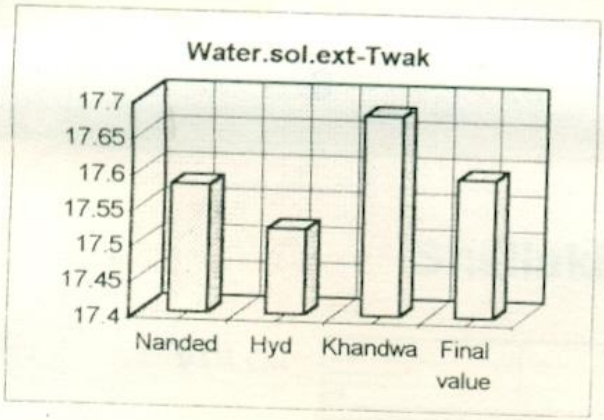
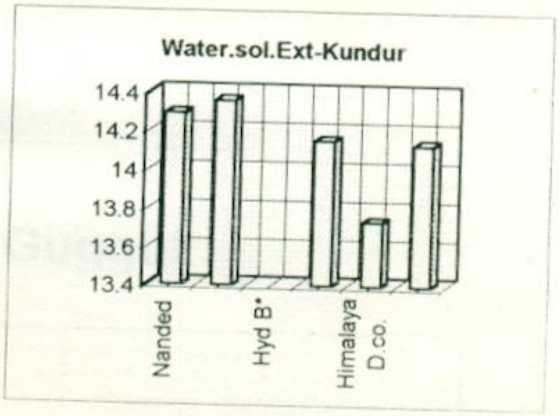

Water insol ash-Twak
4
3
2
1
0
Nanded
Hyd
Khandwa
Final value

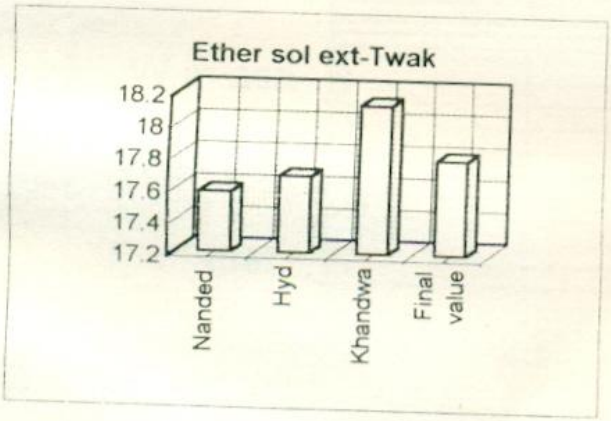

Water insolash-kundur
1.8
1.75
1.7
1.65
Nanded
Hyd [B]
Himalaya

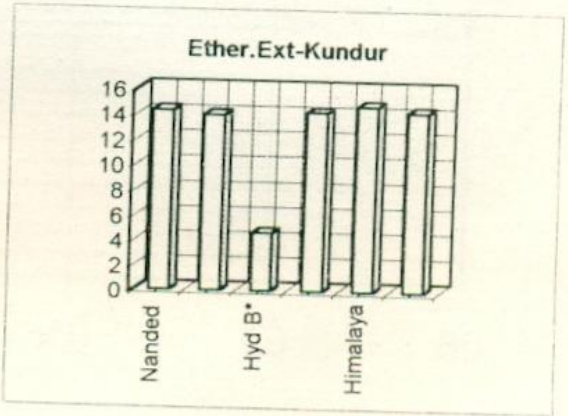

Al.sol.Extractive-Twak
18.8
18.6
18.4
18.2
18
17.8
Nanded
Hyd
Khandwa
Final value


A.Ext.-kundur
66
64
62
60
58
56
54
52
Thomsa forest,
Hyderabad B*
Himalaya D.Co.

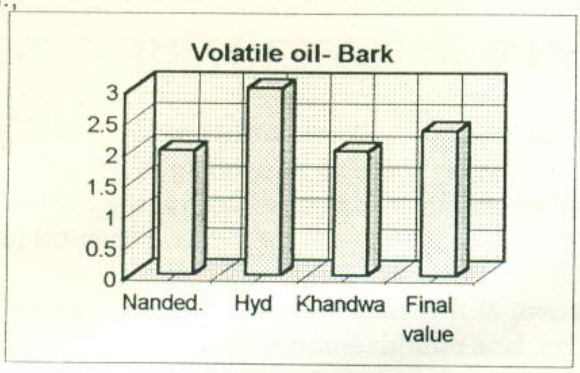

Water.sol.ext-Twak
17.7
17.65
17.6
17.55
17.5
17.45
17.4
Nanded
Hyd
Khandwa
Final value

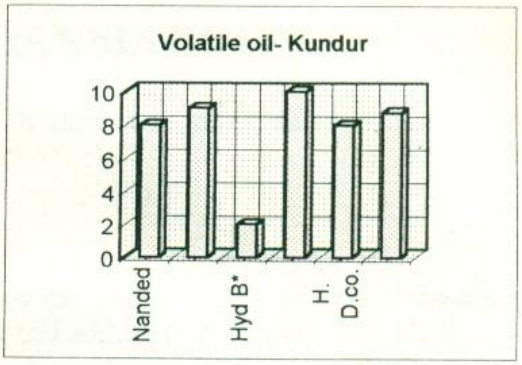

Water.sol.Ext-Kundur
14.4
14.2
14
13.8
13.6
13.4
Nanded
Hyd B*
Himalaya D.co.

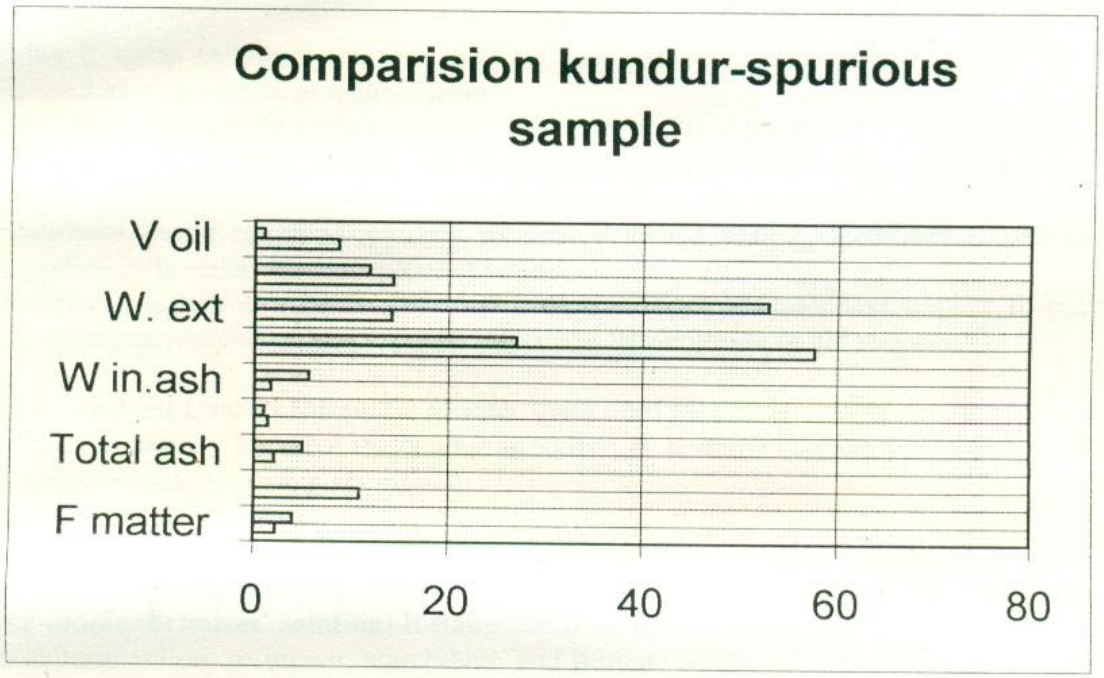

Ether sol ext-Twak
18.2
18
17.8
17.6
17.4
17.2
Nanded
Hyd
Khandwa
Final value

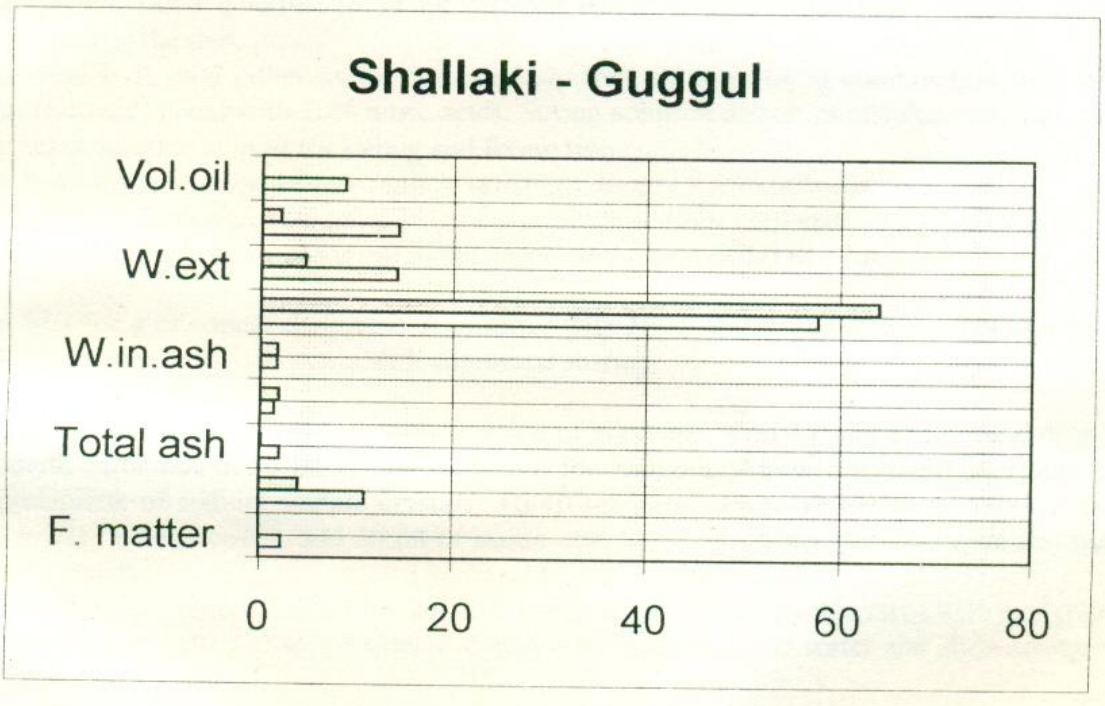

Ether.Ext-Kundur
16
14
12
10
8
6
4
2
0
Nanded
Hyd B*
Himalaya

Contd.,


Volatile oil- Bark
3
2.5
2
1.5
1
0.5
0
Nanded.
Hyd
Khandwa
Final value


Volatile oil- Kundur
10
8
6
4
2
0
Nanded
Hyd B*
H. D.co.


Comparision kundur-spurious sample
V oil
W. ext
W in.ash
Total ash
F matter
0
20
40
60
80


Shallaki - Guggul
Vol.oil
W.ext
W.in.ash
Total ash
F. matter
0
20
40
60
80

REAGENTS USED IN PHARMACOGNOSY

Acetic acid (1-5%) Dilute aqueous solution (1-5%). It does not affect calcium oxalate crystals, however, dissolves globoids of aleurone grains.
Acetone (80%) It is used for extraction of chlorophyll.
Alcohol Ethyl alcohol (95%).

Ammonium vanadate - sulphuric acid It is prepared by dissolving 1.62 g of anhydrous ammonium vanadate in 125 ml of concentrated sulfuric acid, cooling and adding to it 125 ml of ice-cold water. It is used as a spray after ten fold dilution for the detection of alkaloids, phenolic compounds and steroids.

Aniline blue It stains cellulosic tissues. 0.1% aqueous solution stains callus, whereas the alcoholic solution is used as a counter stain with safranin.
Aniline phthalate It is used as a detecting reagent for sugars. It is prepared by dissolving 6 g of aniline hydrochloride in 65 ml of 90% alcohol and 35 ml water and to it add 2 ml concentrated hydrochloric acid.
Aniline sulphate Use a saturated aqueous solution acidified with concentrated sulphuric acid for staining lignified cells walls which it coloured yellow.
Antimony-tri-chloride It is used for the identification of steroids and monoterpene to polyterpene. Dissolve 25 g of antimony-tri-chloride in 100 ml carbon tetrachloride or chloroform.

Canada balsam Heat Canada balsam on a water -bath until be volatile matter is removed and residue becomes hard on cooling. Dissolve the residue in xylene or benzene to from a thin viscous fluid. The reagent is used for making permanent mounts.

Chloral hydrate It is used as a clearing agent. Dissolve 50 g in 20 ml of distilled water. For the removal of chlorophyll, boil material with a few mis of the reagent over flame for a short while.
Chlor-zinc-iodide (Schulzes' solution) It stains cellulose-blue or violet; lignin-yellow;
cutin and suberin-yellow or brown; starch-blue and protein-brown. Dissolve 30 g zinc chloride, 5 g potassium iodide and 1 g iodine in 14 ml distilled water. It should be stored in securely stoppered bottles and kept in the dark.
Chromic acid It is used either as the saturated aqueous solution or in combination with nitric acid (10% chromic acid mixed with 10% nitric acid). Strong solution dissolves cellulose and lignified walls, whereas, weak solution is used for killing and fixing tissues.
Corallin soda It stains callus of sieve-tubes, starch grains and lignified walls, dissolve 5 g of corallinrin in 100 ml of 90% alcohol (a). Dissolve 25 g of crystalline sodium carbonate in 100 ml of water (b). Mix the two solutions when acquired for use in the proportion - 1 ml of (a) to 2 ml of(b).

Cuoxam Mix 0.5 g of copper carbonate in a mortar with 10 ,1 of distilled water and gradually add to it 10 ml of strong solution of ammonia with continued stirring.

Dragendosf Ps reagent It is used for the detection of alkaloids. Boil 14 g of sodium iodide with 5.2 g basic bismuth carbonate in 50 ml glacial acid for a few minutes. Allow it to stand overnight and filter off the precipitate of sodium acetate crystals. To 40 ml water. Preserve the stock solution in amber-coloured bottle. When needed, add 20 ml of acetic acid to 10 ml of this stock solution and make upto 100 ml with water.
Eriich reagent This reagent is used for the detection of ergot alkoloids. Dissolve 0.25 g of p-dimethyl-aminobenxaldehyde with 130 ml of sulphuric acid and 70 ml distilled water and allow to stand for 24 hours prior its use.

Fehling's solution It is used for detection of reducing sugars. Dissolve 34.66 g of copper sulphate in distilled water and make volume upto 500 ml (Solution A). Dissolve 173 g of potassium sodium tartarate and 50 g of sodium hydrate in distilled water and make volume upto 500 ml (Solution B). Mix two solutions in equal volume prior to use.

Ferric chloride(alcoholic) A 5% w/v solution of ferric chloride in 90% alcohol and used for detection of phenols.

Fronde's reagent It gives bluish green colour with ipecacuanha alkaloids. Dissolve 1 g of sodium molybdate in 100 ml concentrated sulphuric acid and keep for a few weeks prior to use.
Glycerin solution 50% aqueous glycerin solution used as a temporary mountant.

Iodine water It is used for the detection of starch grains (blue); cellulose, proteins and lipids (yellow to brown). Dissolve 2 g of iodine and 3 g of potassium iodine and add about 5 ml of water. Agitate until dissolved. Slowly dilute to make 100 ml with water. Store in a well fitted and closed container.
Lactophenol Dissolve 20 g each of phenol and lactic acid, 40 g of glycerin in 20 ml distilled water. It is used as a mountant for temporary preparations especially for unorganized drugs, such as starch.
Lead acetate A 25% basic lead acetate solution is used for the detection offlavonols.
Liebermann Burchard reagent Mix 10 ml of acetic anhydride with 10 ml of concentrated sulphuric acid under cooling. To this add, 100 ml of absolute alcohol under cooling. The reagent is used for detection oftriterpenes and steroids.

Mayor's reagent It is used for the detection of alkaloids. Dissolve 1.36 g of mercuric chloride in 60 ml distilled water (a). Dissolve 5 g of potassium iodide in 20 ml distilled water(b). Mix (a) and (b) and adjust the volume to 100 ml with distilled water.
Millon's reagent It is a detecting reagent for proteins. Dissolve 1 g of mercury in 9 ml of fuming nitric acid, keeping the mixture well cooled during the reaction. When the action is complete, add equal volume of distilled water.

Molisch's reagent Dissolve lOg ofa-naphthol in 100 ml of 95% alcohol. It is used for detection of carbohydrates.

Ninhydrin reagent It is used for detection of amino acids. Prepare 0.1% solution in *n*-butanol.

Phloroglucinol solution Dissolve 2 g of phloroglucin in 100 ml of 90% alcohol. It is a staining reagent along with concentrated hydrochloric acid for lignin and liquefied tissues.

Ruthenium red It is used as a staining reagent for mucilage. In 10 ml of 10% solution of lead acetate, dissolve 0.008 g of ruthenium red. Use freshly prepared solution.

Safranin Make 1% solution of safranin in 50% alcohol. It is used as a counter stain in double staining. It is retained by lignified, suberized and cutinised cell walls.

Sudan Red III It is a staining reagent for oil, cuticle and suberin. Dissolve 0.04 g of Sudan red in 20 ml of alcohol (90%) and 20 ml glycerin. Alternatively a saturated solution of the dye in 70% alcohol after warming to dissolve can be use

Tannic acid solution Dissolve 5 g oftannic acid in distilled

Vanillin-sulphuric acid It is used as a spraying reagent for detection of steroids and terpenes. The plates are sprayed with the reagent and heated at 100°C for 5 minutes. It is a freshly prepared solution of 0.5% vanillin in sulphuric acid ethanol (4 : 1).

Wagner's reagent It is a general reagent for detection of alkaloids. Dissolve 1.27 g of iodine and 2 g of potassium iodide in 5 ml of water and make up the volume to 100 ml with distilled water.

Formats of Various Countries for Presenting the Monographs

1. British Herbal Pharmacopoeia

Monograph title
Synonyms
Definition
Description
Macroscopic
Microscopic
Physico-Chemical Parameters
Moisture content
Total ash/ Acid insoluble ash/ water Sol. Ash/ foreigh organic matter/ contents of Stalks/ other- Fruit & Seeds
Contents of Alkaloids/ Volatile Oil
TLC/ GLC/ PC/ Electrophoresis Ref.
Identification test (Color reactions)
Biological ativity Related Parameters
Bitterness/ Absorbency (Selling Index)
Therapeutics
Action
Indication
Specific indication
Combinations used
Precautions
Preparations and Maximum Concentration
Toxicity
Incompatibilities
Contraindications
Directions for use
Side effects
Storage

2. Japanese Standards For Herbal medicines [JSHM]

Title:
Botanical name:
Definition:
Description:
Macroscopic:
Microscopic:
Organoleptic:
Simple Physico- Chemical Tests (Solubility, Ph etc.,)
Identification Tests:

contd.,

Purity: By means of rests for Absence of Adulteratants/ Impurities/ Undesirable Properties/ Foreign Matter.
LOD:
Total Ash/ acid Insol. Ash:
Extract Content in Water / Ethanol/ Ether:
Essential Oil:
Storage:

3. Ayurvedic pharmacopoeia Of India (PI)- Monograph

Monograph: Title: Classical Sanskrit Name
Definition: Scientific name, occurrence,distribution, collection
Synonyms: Names in Sanskrit, English, Dhindi, Urdu, and other Indian Languages
Description: Macroscopic: gross external morphology of intact drug.
Microscopic: For sections of intact drug and particles of powdered drug.
Identity , Purity. And Strength.
Identification tests: Color/ ppt reactions
Foreign matter: only upper limit
Acid insol. Ash: Only upper limit
Alcohol Soluble extractive: only lower limit
Assay: total alkaloids: total volatile oils
Constituents: Only for information, not as standard for conformity
Classicial attributes: Rasa, Guna. Virya, Vipaka, And Karma (with out any standard methods to identify or quantify them)
Therapeutic Uses: As per Ayurvedic classic.
Important formulations: As per Ayurvedic Classics
Doses: oral adult dose in metric system
(by appprox. Conversion from classical weights mentioned in Ayurveda Texts)

Bibliography

1. Ravi Dutta Tripathi, Astanga Sangraha- Sutra Sthan.3rd Ed., Chaukhamba Sanskrit Pratisthan, New Delhi, 1993.
2. Bhavamisra, Bhavaprakasha (first part), Kashi Sanskrit series 130, 8th ed. Chaukhambha Sanskrit sansthan, PO Box no. 1139, K37/116, Gopal Mandir lane Varanasi, 1993.
3. Charaka Samhita with Ayurveda Deipika commentary, Chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint,1992
4. Acharya Shri Radhakrishna Parashar, Sharangadhara Samhita,with Hindi trans., Baidyanath Ayurved Bhavan, 1984.
5. Susruta Samhita with Nibandha Sangraha commentary, chaukhambha Surabharati Prakashan, K 37/177, Gopal Mandir lane, PO box. No. 1129, Varanasi-1, reprint, 1994.
6. Shivakosha of sivadatta Mishra (Ed.). Harshe, R.G. Deccan College and Postgraduate Research Institute Poona, 1952.
7. Pandit. Jagannatha sharma Bajpeyee, Chakradatta 4th ed. Sri Laxmi Venkateswera steam press, Kalyan Mumbai, 1959.
8. Sureshchandra Banarji, Flora and fauna in Sanskrit Literature, NyaProkash, 206, Bidhan Sarini, Calcutta 700006.
9. Pandit Narahari., Raja nighantu., Krishnadas Academy, Varanasi, 1882 I ed.
10. Dhanvantari Nighantu., Chowkhamba Orientalia,varanasi. II ed., 1998.
11. Ganga Vishnu Shrikrishna das., Madanapal Nighantu, Lakshminarayana Press, Kalyan, Mumbai-1954 .
12. P.V.Sharma, Ed., Kayyadeva Nighantu (Pathyapathya Vibodhaka), Chaukhambha Orientalia, Varanasi, I Ed. 1979 .
13. Bapalal G. Vaidya, Nighantu Adarsh vol-I, V.Ayurveda series 54, Chaukhambha Bharati Academy, Gokul Bhawan, k. 37/109, Gopal Mandir lane, PO. Box no.1065, Varanasi-1, First ed.
14. M. Abdul Karem, Plants in Ayurveda, FRLHT, No. 50, MSH, Layout, Anandnagar,Bangalore,1997.
15. Dr. K.H.Krishnamurthy., The wealth of Susruta, International Institute of ayurveda,Coimbatore, 1991.
16. Krishnamurthy, K.H. Botanical identification of ayurvedic medicinal plants; a new method of Pharmacolinguistics. A preliminary account –Medicinal plants 1.MSS Information Corporation, New York, 1974.
17. Krishnamurthy K.H An evaluation of Ayurvedic pharmacognosy. J. Indian Pharm. Manuf.
18. Krishnamurthy K.H. Botanical aspects of the Triphala drugs of Ayurveda. Indian Journal m Med., Res 5, 1, 1970.
19. Bole, P.V. Botanical drugs of indigenous medicinal practices. Some problems and perspectives. J/ Indian Pharm.Manuf., 1966.
22. Dufrene, H.La flora Sanscrite. Essai d' explication dies noms sanscrits servant a designer les principales plante de a' inde d "pre's leur etymologic, Paris.
23. Filliozat, Jean. La doctrine Classique de la Medicine indicine indienne: Ses origines et ses paralleles grecs, University of Paris, 1949.
24. Jain, S.K the origin and utility of some vernacular plant names. Proceedings of the National Academy of Science of India, 83, 525, 1963.
25. A barefoot doctor's Mannul. Prepared by the revolutionary Health committee of Hunan Province. Routledge and Kegan Paul, London and Henely, 1978.
26. C.K.Kokate, A.P. Purohit, S.B.Gokhale., Pharmacognosy , Nirali prakashan, 19th ed.,2002.
27. The Ayurvedic Pharmacopoeia of India, vol-1, part-II, Dept. of ISM, Ministry of Health and Family Welfare, Govt. of India, 1999.
28. Anonymous., Manual for Quality control of Herbal Drugs, National Botanical Research Institute, Lucknow-1, 2001.
29. Trease & Evans Pharmacognosy, Wb Saunders Company Ltd.
30. Dr. M.D. Nair, Standardization imbroligo effects traditional medicinal products, Heritage Healing, Sep 2000, healthcare communications(p) ltd. #8, 5th cross Extn. Bhaktavathsalam Nagar, Adayar, Chenni-20 (India.)

[Notification: Ministry of health and family welfare, dept. of ISM, Jan 23, 2000-amendment to Drugs & Cosmetics rules, 1945 (rules 155B-157B-schedule-T).

31. C.K.Katiyar,A.Vittal babu,D.B.Narayana-Standardization of ayurvedic drugs-Problems,Realities and rationale; dabur Inida Ltd., Dabur Research foundation.,2,site-iv,Sahibabad,Ghaziabad-201 010 (U.P).-Traditional medicine.,Oxford &IBH co.Pvt.Ltd. 1993.,p.328
32. Mrs.Shailaja Chandra.,Forward.,The Ayurvedic Pharacopoeia of India- III,NISCOM,New Delhi,1999.
33. C.K.Katiyar,A.Vittal babu,D.B.Narayana-Standardization of ayurvedic drugs-Problems,Realities and rationale; dabur Inida Ltd., Dabur Research foundation.,2,site-iv,Sahibabad,Ghaziabad-201 010 (U.P)
34. Biswapati Mukharjee., Traditional medicine.,Oxford &IBH co.Pvt.Ltd. 1993.,
35. G.B. Singh et.al., Anti-inflammatory action of boswellic acids, Phytomedicine, vol-3, Gustav Fisher Verlag, Stuttgart, Jena, Newyork, 1996.
36. Asima Chatarjee., Satyesh Chandra Prakash., The treatise of Indian medicinal Plants, Vol 3, NISCOM, New Delhi, 1997.
37. Ram P. Rastogi., B.N. Mehrotra., Compendium of Indian Medicinal Plants – vol 1-6 Central Drug research Institute , Lucknow 7 CSIR- N. Delhi- 1998, [NISCOM].
38. Rolla Sheshagiri Rao.,Flora of East Godavari Dist. Indian National Trust for Art and Cultural Heritage,Hyd. A.P and [INTACH, New Delhi].
39. V.Singh, R.P Randey., Ethnobotany of Rajasthan, India Scentific Publications, Jodhpur I ed., 1998.
40. Ed. S.K Jain & R.R Rao., An assessment of threatened plants of India ., Howrah., 1983.
41. Anonymous; The wealth of India, Vol; 2 B, Publications and information Division, ICMR, N. Delhi, 1988.
42. R.A Davis, Taber's Cyclopedic Medical Dictonary, 17th ed. 1989. Phytochemical Studies of Unani formulations, Part- 2 CCRUM, Govt India, N. Delhi., 1987.
43. Shri Viswanath Dwivedi, Kriyatmak Aushadhaparichaya Vigyan, , Chaukhamba Vidya Bhavan, Varanasi 1966.
44. T. Pullaiah, P. Surya prakash Babu, Flora of Andhrapradesh, Scientific publishers, Jodh pur, India, 1998.
45. The useful plants of India, Publications and Indirmation Directorate, CSIR, Dr. K.S. K Marg, N. Delhi- 1994(Reprint)
46. Purudhottam Kaushik, Anil Kumar Dhimon, Medidinal plants & Raw materials of India; Bishen Shingh , Mahendrapal Singh, 23 – A, Connaught place, P.).B no. 137, Dehradun, India 2000.
47. Wiilliam Dyock , Pharmacographia indica .,Hamdard. The orgaon of the Institute of Health and Tibb research, - Hamdard national foundation, Pakistan, 1972
48. S.C. Dutta, Systematic Botany, 5th Ed. New Age International Ltd. N. Delhi, 1996.
49. John. H. Weirsema, Blanca Leo'n. CRC Press, World economic plants – a standard reference- Washington DC, 1999.
50. Aiyer, K.N., Nambodiri, A.N., & Kolammal. M, 1957, Pharmacognosy of Ayurvedic Drugs .Vol.3 Trivendram.
51. Alix Taisom., 1974, La- Harita Samhia, French Institute, Pondicherry.
52. Anonymous- 1951, Pharmacognosy of Ayurvedic Drugs. Vols. 1 & 3 Trivendrum
53. Anonymous- 1978. Ayurvedic formulary of India part I controller of Publications, New Delhi.
54. George Watt., 1989., A Dictionary of the Economic Products of India, Vol I-VA Calcutta
55. Kirtikar & Basu, 1935. Indian Medicinal Plants (Revised By Blatter, Acius & Mhaskar) 2nd Ed. 5. Vols. (with (1033 plates) Lalit Mohan Basu, Allahabad.
56. Kurup, P.n.V et al 1968., Handbook of medicinal plants (revised & enlarged) CCRAS, New Delhi.

57. Modern Sheriff., 1988, . A Catalogue of Indian Synonyms, Delhi
58. Mooss, N.S., 1976. Single Drug Remedies, Kottayam.
59. Mooss, N.S 1980, Ganaas of Vahata, Vaidyasarathy Press, Kottayam
60. Mukerji, B., 1953, Indian Pharmaceutical Codex. Vol I, New Delhi
61. Nadkarni , 1953, Indian Materia Medica 2 vols. Popular Book Depot, Bombay.
62. Raghnath Iyer, 1953- 96. Inidan Medicinal plants vol 1-5, Madras.
63. Sharma PV ., 1994. Dravyaguna Vignana, vol 2& 5 Chowkambha,Varanasi.
64. Sivarajan, V.V& Indira Balachandran, 1994, ayurvedic Drugs and Their plant sources, New Delhi.
65. Thakur Balwant Singh & Chunekar, K.C, 1972, glossary of Vegetable Drugs in Brihatrayi, Varanasi.
66. Vaidya Bapalal, 1982, Some Controversial Drugs of India, Varanasi
67. Vaidya Kamat, D.K 1972 Studies on Meidicinal Plants in Dhanvantariya Nighantu Vol. I Poona.
68. Whitelaw Ainslie., 1984, Materia Indica, Delhi.
69. General guidelines for methodologies on research and evaluation of traditional medicine WHO, Geneva, 2000.

List of Web-sites Visited for information:

1. www. Naturesformulary .com
2. www. Modern-natural.com
3. www. himalayahealthcare.com
4. www. Ayurvedicsuppliments.com
5. www.himalayadrugco.com
6. www. Google.com
7. MEDLARS-NIC, N. Delhi.

The ultrastructure of epithelial cells of loeo-gumresin ducts in Boswellia serrata, the source of indian olibanum [IF] is described.

D. Phyto-chemistry

1. Fattorusso-E; santacroce-C; xaasan-CF;4(23)- dihydroroburic acid from the resin (incense) of Boswelliacarterii [b.sacra],National Somali university, p.o. box 1081, Mogadishu, Somalia.Phytochemistry. 1983,zz:12,z868-z869;

2. Sharma-ra; varma-kc; Studies on gum obtained from boswellia serrata roxb.Dep. Pharmaceutical sci., univ. saugar, sagar, India; Indian-drugs. 1980, 17:8,225-227;
chemical properties are reported of salai gum. Compared with
tragacanth and CMC the gum is not a good suspending agent but
emulsions prepared with it were slightly better than those from acacia.

3. Wahab-sma ; aboutabl-EA; E1-zalabani-SM; fouad-HA; pooter-hl-de; E1-fallaha-B;the essential oil of olibanum.Faculty of pharmacy, cairo university , 11562 cairo, Egypt.Planta-medica. 1987, 4, 382-384;

the essential oil of the oleogum resin from the bark of boswellia carterii[b.sacra]
was prepared by direct steam distillaton and also by steam distillation of an n-hexane extract. Thirty-three components were identified; 11 of these (all minor constituents)were not found in the chromatogram of the oil from the n_hexane extract.the oils contain 62.1% easters, 15.4%easters alcohols, 9.9% monoterpene hydrocarbons and 7.1% diterpenes. Both oil preparations showed antimicrobial activity in screening tests,(that prepared by direct distillation being the more effective.) These findings support the use of the resin in local medicine to treat coughs and asthma.

4. Retamar-JA.IPNAYS(CONICET-UNL), chemistry and technology of natural and synthetic aromas.Santigo del estero z8z9,3000 santa Fe,Argentina, Monographias-de-la- academia-national-de-ciencias-exactas,-fisicas-y-naturales,-buenos-aries.1988,no.3,55-69;

		Average				1.49
3.	Hyderabad [B]	i.	10.2360	10.2620	0.026	2.6
	(Gold colored sample	ii.	10.2362	10.2621	0.0259	2.5
		iii.	10.2620	0.0261	0.0261	2.61
		Average				2.56
4.	Jaipur Market	i.	11.7966	11.8160	0.0194	1.9
		ii.	11.7966	11.8158	0.0192	1.9
		iii.	11.7964	11.8155	0.0191	1.91
		Average				1.9
5.	Sample of Himalaya drug Co.	i.	10.2361	10.2501	0.0145	1.45
		ii.	10.2361	10.2505	0.0143	1.43
		iii.	10.2362	10.2505	0.0141	1.41
		Average	10.2362	10.2505	0.0143	1.43
	Final Value					1.4

5. DETERMINATION OF WATER INSOLUBLE ASH

The ash obtained as above is boiled for 5 minutes with 25 ml of water; then the insoluble matter is collected in a crucible, on an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°. The value obtained here is the water insoluble ash.

Subtracting the weight of the insoluble matter from the weight of the total ash; represents the water-soluble ash.

Table no. 9 Water Insoluble Ash of Bark Powder

S.No	Place of collection	S.No.	Weigh of the crucible (before)-W1	Weigh of the crucible (after)-W2	Weight of the Ash=w2-w1	Ash in %
1.	Nanded	i.	11.7965	11.8286	0.0321	3.21
		ii.	11.7966	11.8284	0.0322	3.22
		iii.	11.7965	11.8286	0.0321	3.21
		Average				3.2
2.	Hyderabad	i.	10.2387	10.2697	0.0310	3.1
		ii.	10.2386	10.2700	0.0320	3.2
		iii.	10.2385	10.2737	0.0350	3.5
		Average				3.1
3.	Khandwa, M.P	i.	10.2387	10.2775	0.0388	3.8
		ii.	10.2386	10.2774	0.0388	3.8
		iii.	10.3287	10.2774	0.0388	3.8
		Average				3.8
	Final value					3.36%